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Inhibitory Effects of Herbal Medicines on the Platelet-Activating Factor (PAF) Receptor Binding

Young-Hwa Kang*

College of Agricultural and Life Science, Kyungpook National University, 1370 Sankyuk-dong, Buk-gu, Daegu 702-701, Korea

Abstract – Methanolic extracts of ninety-five medicinal plants were screened for platelet-activating factor (PAF) receptor binding inhibitory activity using rabbit platelet. *Alpinia officinarum, Belamcanda chinensis, Leonurus heterophyllus, Pinus densiflora, Polygonatum sibiricum* and *Sambucus williamsii* showed significant inhibitory effects on the platelet-activating factor (PAF) receptor binding.
Keywords – Platelet-Activating Factor (PAF) receptor binding inhibitory effects, Herbal medicines

Introduction

Platelet-activating factor (PAF) is a potent glyceropho-pspholipid mediator (Maller *et al.*, 1985) of inflammation and allergy that plays a wide range of physiological and pathological roles (Braquet *et al.*, 1987; Hanahan *et al.*, 1987). It is involved in a variety of clinical conditions such as bronchial asthma, endotoxin shock, pulmonary dysfunction, pancreatitis (Curtis, 1996), allergy, cardiac anaphylaxis (Vargaftig *et al.*, 1987), thrombosis (Kloprogge *et al.*, 1983), gastrointestinal ulceration, transplanted organ rejection, ovoimplantation in pregnancy and corneal diseases (Hsueh *et al.*, 1986; Ito *et al.*, 1984). PAF is generated in inflammatory cells such as eosinophils, neutrophils, alveolar macrophage, and platelets by immune stimuli. PAF binds to PAF receptors and triggers in a series of biological responses including platelet activation, bronchoconstriction, ulcerogenesis and hypotension (Koltai *et al.*, 1991; Snyder, F., 1985).

As part of continuous screening studies to identify the novel PAF antagonist (Han *et al.*, 1995; Jantan *et al.*, 1996; Fan *et al.*, 2001), PAF receptor binding inhibitory effects of ninety-five medicinal plants were evaluated by PAF receptor binding assay using rabbit platelet.

Experimental

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

Plant material and extraction – The plant materials were purchased from herbal drug store at Seoul, Korea. The air-dried materials were extracted with methanol 3 times. The methanol extracts were concentrated under reduced pressure to give viscous mass. The methanol extract of *Ginkgo biloba* was used as positive control.

Reagents – Tris-tyrode buffer (0.01 M, pH 7.3) was used for washing of platelets and binding studies. Acid citrate dextrose (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, Amersham, UK) was dissolved in tris-tyrode buffer containing 0.25% BSA.

Preparation of samples for PAF receptor binding assay – Samples were dissolved in dimethyl sulfoxide (DMSO) and diluted with saline (final concentration of DMSO, 0.2%). 0.2% DMSO solution in saline was used as control. Preliminary test confirmed that 0.2% DMSO does not interfere with the receptor binding studies.

PAF receptor binding assay – PAF receptor binding assay was carried out according to the method of Valone (Valone *et al.*, 1982) with some modification. In brief, six volumes of blood were collected from the heart directly into 1 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, and the obtained platelets were then washed

*Author for correspondence
Fax: +82-53-950-5722; E-mail: youngh@knu.ac.kr

Table 1. Inhibitory effects of the methanol extract of herbal medicines on the PAF receptor binding

Species	Family	Part used	% Inhibition[a), b)]
Achyranthes bidentata	Amaranthaceae	Leaf, Stem	–
Adenophora verticillata	Campanulaceae	Leaf, Stem	45
Agastache rugosa	Labiatae	Leaf	12
Aloe ferox	Asphodelaceae	Leaf, Stem	32
Alpinia officinarum	Zingiberaceae	Root	64
Alpinia oxyphylla	Zingiberaceae	Fruit	32
Amomum xanthioides	Zingiberaceae	Fruit	15
Anemarrhena asphodeloides	Liliaceae	Root bark	17
Angelica gigas	Umbelliferae	Root	–
Anthoxylum nitidum	Rutaceae	Root	47
Aquilaria agallocha	Thymelaeaceae	Stem	–
Ardisia japonica	Myrsinaceae	Leaf, Stem	39
Arca inflata	Arcidae	Shell	30
Areca catechu	Palmae	Seed	25
Arecae pericarpium	Palmae	Fruit	15
Aristolochia contorta	Aristolochiaceae	Stem, Leaf	20
Artemisia anomala	Compositae	Whole plant	–
Astragalus membranaceus	Leguminosae	Root	–
Atractylodes japonica	Compositae	Rhizome	–
Belamcanda chinensis	Iridaceae	Stem, Leaf	50
Boschniakia rossica	Orobanchaceae	Whole plant	18
Chirysanthemi zawadskii	Compositae	Leaf	40
Cinnamomum cassia	Lauraceae	Stem	20
Cinnamomum loureirii	Lauraceae	Stem bark	–
Citrus tangerine	Rutaceae	Fructus bark	25
Cornus officinalis	Cornaceae	Leaf	–
Crysanthemum morifolium	Compositae	Stem, Leaf	10
Cuscuta chinensis	Convolvulaceae	Seed	–
Cyperus rotundus	Cyperaceae	Leaf	18
Cynanchum stauntoni	Asclepiadaceae	Stem, Leaf	14
Dalbergia odorifera	Leguminosae	Root	44
Datura metel	Solanaceae	Stem, Leaf	46
Dianthus chinensis	Caryophyllaceae	Flower	–
Dictamus albus	Rutaceae	Stem	45
Dimocarpus longan	Sapindaceae	Fruit	29
Dryobalanops aromatica	Dipterocarpaceae	Fruit	17
Dryopteris crassirhizoma	Polypodiaceae	Root	–
Drynaria fortunei	Polypodiaceae	Rhizome	31
Eclipta prostrata	Compositae	Whole plant	45
Elsholtzia splendens	Labiatae	Leaf	40
Erycibe obtusifolia	Rosaceae	Leaf	–
Eucrium veronicoides	Labiatae	Leaf	–
Eugenia caryophyllata	Myrtaceae	Flower	31

Table 1. continued

Species	Family	Part used	% Inhibition[a), b)]
Euphobia lathyris	Euphorbiaceae	Leaf	27
Foeniculum vulgare	Umbelliterae	Leaf, Stem	16
Forsythia suspensa	Oleaceae	Seed	–
Forsythia viridissima	Oleaceae	Stem, Leaf	–
Fritillaria thunbergii	Liliaceae	Stem	–
Gentiana macrophylla	Gentianaceae	Root	34
Ginkgo biloba	Ginkgoaceae	Leaf	80
Glycine max	Fabaceae	Seed	19
Glycyrrhiza glabra	Leguminosae	Root	24
Houttuynia cordata	Saururaceae	Root	21
Impatiens balsamina	Balsaminaceae	Seed	48
Impatiens balsamina	Balsaminaceae	Stem, Leaf	46
Juncus effuses	Juncaceae	Stem, Leaf	14
Leonurus heterophyllus	Labiatae	Leaf	51
Lepidium apetalum	Cruciferae	Seed	31
Lilium brownii	Liliaceae	Leaf	24
Ligustrum lucidum	Oleaceae	Root	21
Lindera strychnifolia	Lauraceae	Root	41
Lycium chinense	Solanaceae	Root bark	32
Magnolia obovata	Magnoliaceae	Stem, Leaf	–
Magnolia officinalis	Magnoliaceae	Stem bark	–
Malva verticillata	Malvaceae	Seed	16
Momordica cochinchinensis	Cucurbitaceae	Fruit	40
Morus alba	Moraceae	Leaf	–
Omphalia lapidescens	Ployporaceae	Sclerotium	48
Osmunda japonica	Osmundaceae	Leaf	–
Paeonia lactiflora	Paeoniaceae	Stem, Leaf	16
Paris petiolata	Liliaceae	Root	–
Patrinia villosa	Valerianaceae	Whole plant	–
Phytolacca acinosa	Phytolaccaceae	Root	–
Picrorrhiza kurroa	Scrophulariaceae	Rhizome	10
Pinus densiflora	Pinaceae	Leaf	60
Pogostemon cablin	Libiatae	Leaf	–
Polygonatum sibiricum	Liliaceae	Root	51
Polygonum cuspidatum	Polygonaceae	Root	42
Poncirus trifoliate	Rutaceae	Fruit	40
Poria cocos	Polyporaceae	Sclerotium	19
Prunus armeniaca	Rosaceae	Seed	30
Rehmannia glutinosa	Scrophulariaceae	Rhizome	11
Rosa rugosa	Rosaceae	Seed	20
Sagittaria sagittifilia	Alismataceae	Leaf	23
Sambucus williamsii	Caprifoliaceae	Stem	56
Santalum album	Santalaceae	Stem	–
Sesamum indicum	Pedaliaceae	Seed	10
Spirodela polyrrhiza	Lemnaceae	Leaf	30
Stephania tetrandra	Menispermaceae	Root	18

Table 1. continued

Species	Family	Part used	% Inhibition[a), b)]
Taraxacum mongolicum	Compositae	Whole plant	21
Tricosanthes kirilowii	Cucurbitaceae	Seed	15
Vitex rotundifolia	Verbenaceae	Fruit	10
Vladimiria souliei	Cucurbitaceae	Root	12
Xanthium stramonium	Compositae	Fruit	–
Zizyphus jujuba	Rhamnaceae	Fruit	–

a) Concentration: 2 mg/10 ml
b) -; less than 10%

three times by centrifugation (900 g, 10 min.) in tris-tyrode buffer. The final platelet concentration was adjusted to 3× 10^8 platelets/ml in tris-tyrode buffer containing 0.25% BSA by means of a platelet counter.

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 counter. 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

In order to evaluate the inhibitory potential of herbal medicines on the platelet activating factor (PAF) receptor binding to rabbit platelet, ninety-five medicinal plants were investigated. Inhibitory effects of MeOH extracts were summarized in Table 1. The MeOH extracts of six plants, *Alpinia officinarum, Belamcanda chinensis, Leonurus heterophyllus, Pinus densiflora, Polygonatum sibiricum* and *Sambucus williamsii* showed significant inhibitory effects of more than 50% at a concentration of 200 μg/ml. Two most active plants, *Alpinia officinarum* and *Pinus densiflora,* were extracted with hexane, and then the residues were extracted with chloroform and methanol, successively. The inhibitory effect of PAF receptor binding of each fraction was evaluated. As shown in Table 2, the hexane fractions of *Alpinia officinarum* and *Pinus densiflora* showed more potent inhibitory activities than other fractions.

Table 2. Inhibitory effects of the hexane, chloroform, and methanol fractions of *Alpinia officinarum* and *Pinus densiflora* on the PAF receptor binding

Species	Part used	% Inhibition[a), b)]		
		Hexane	$CHCl_3$	MeOH
Alpinia officinarum	Root	65	20	–
Pinus densiflora	Leaf	50	31	–

a) Concentration: 2 mg/10 ml
b) -: less than 10%

Table 3. Inhibitory effects of the hexane fractions of *Pinus densiflora* on the PAF receptor binding

Species	% Inhibition[a), b)]						
	F1	F2	F3	F4	F5	F6	F7
Pinus densiflora	–	–	31	45	57	62	36

a) Concentration: 2 mg/10 ml
b) -: less than 10%

Pinus densiflora has been used traditionally to treat inflammation (Choi, 1991). The hexane fraction of *Pinus densiflora* was therefore subjected to silica gel column chromatography. Among seven fractions, the sixth fraction exhibited potent inhibitory effect (62% inhibition at a concentration of 200 μg/ml) as shown in Table 3. The isolation and characterization of active compounds remained to be further investigated.

In conclusion, the preliminary screening study of herbal medicines shows that *Alpinia officinarum, Belamcanda chinensis, Leonurus heterophyllus, Pinus densiflora, Polygonatum sibiricum* and *Sambucus williamsii* are potential sources of novel PAF antagonists.

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