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Evaluation of Inhibitory Potentials of Chinese Medicinal Plants on Platelet-Activating Factor (PAF) Receptor Binding

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Abstract – Methanol extracts of eighty Chinese medicinal plants were investigated for platelet-activating factor (PAF) receptor binding inhibitory activity using rabbit platelet. Extracts of *Cratoxylon ligustrinum*, *Kalimeris indica*, *Euonymus japonica*, *Ophiopogon japonicus*, *Gleditsia sinensis*, *Clausena lansium*, *Agave sisalana* were found to exhibit significant inhibitory effects. Chloroform partition of the Methanol extract of *Kalimeris indica* was further fractionated by column chromatography to afford one strong active subfraction with 93.6% inhibition at a concentration of 100 μg/ml.
Key words – platelet-activating factor (PAF) receptor binding inhibitory activity, Chinese medicinal plants

Introduction

Platelet-activating factor (PAF) is an endogenous phospholipid inflammatory mediator, which has a D-glycerol skeleton bearing a phosphorylcholine at C_3, an acetyl group at C_2, and a long-chain alkyl ether moiety at C_1. PAF plays a wide range of physiological and pathological roles. Inflammatory cells such as alveolar macrophage, eosinophils, platelets, and neutrophils generate PAF in response to inflammatory and immune stimuli. PAF binding to PAF receptors then results in a series of biological responses including increased vascular permeability, hemoconcentration, hypotension, ulcerogenesis, bronchoconstriction, triggering of airway hyperresponsiveness, and platelet degranulation. These proinflammatory activities indicate that PAF could be an important mediator in wide range of pathological conditions which would include septic shock, asthma, ischemia/reperfusion injury, pancreatitis, inflammatory bowel disease, and rhinitis (Barquet *et al.*, 1987; Summers *et al.*, 1995).

As part of our continuous screening studies to identify novel PAF antagonists from tropical plants (Han *et al.*, 1994; Han *et al.*, 1995a, b; Ibrahim *et al.* 1996), 80 species of Chinese medicinal plants which have been used to treat PAF related diseases were collected and evaluated for their PAF receptor binding inhibitory effects. Herein we report our preliminary screening results.

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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 material and extractions – 80 species of Chinese medicinal plants were collected from Guangxi Botanical Research Institute (GBRI) of P. R. China in 1998. The botanical identification was conducted by Dr. Guo Jixian. The voucher specimens have been deposited in GBRI. Each plant was air-dried and extracted with methanol 3 times. The methanol extract were concentrated under reduced pressure and further dried under vacuum to give viscous mass.

Reagents and buffers – Tris-tyrode buffer (0.01 M, 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, Amersham, UK) was dissolved in tri-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%). Control test was carried out with 0.2% DMSO in saline solution

instead of sample solution. Preliminary test confirmed that 0.2% DMSO does not interfere with the receptor binding studies.

Preparation of washed rabbit platelet suspension - 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 carefully removed. PRP was recentrifuged at 750 *g* for 10 min, and the obtained platelet were then washed three times by

Table 1. Inhibitory effects of Chinese medicinal plants on the platelet-activating factor (PAF) receptor binding

No.	Species	Part used	% Inhibition
01	*Selaginella uncinata*	whole	3.9
02	*Ptarideum aquilinum*	whole	12.1
03	*Humata tyermanni*	whole	23.0
04	*Drynaria fortunei*	whole	26.7
05	*Podocarpus macrophyllus*	branches and leaves	25.7
06	*Nandina domestica*	branches and leaves	–
07	*Michelia alba*	branches and leaves	48.4
08	*Michelia champaca*	branches and leaves	46.2
09	*Mirabilis jalapa*	whole	23.2
10	*Melastoma candidum*	whole	34.4
11	*Cratoxylon ligustrinum*	branches and leaves	54.3
12	*Euphorbia hirta*	whole	32.5
13	*Agrimonia pilosa*	whole	35.0
14	*Rubus alceaefolius*	whole	29.4
15	*Ormosia henryi*	branches and leaves	34.8
16	*Murraya paniculata*	branches and leaves	32.1
17	*Rhus chinensis*	branches and leaves	–
18	*Jasminum amplexicaule*	branches and leaves	28.7
19	*Rauvolfia verticillata*	branches and leaves	42.1
20	*Uncaria rhynchophylla*	branches and leaves	–
21	*Bidens pilosa*	whole	36.9
22	*Erigeron Canadensis*	whole	30.7
23	*Gynura crepidioides*	whole	34.9
24	*Kalimeris indica*	whole	53.5
25	*Senecio scandens*	whole	–
26	*Parbitis nil*	whole	19.7
27	*Paulownia fortunei*	branches and leaves	31.2
28	*Radermachera sinica*	branches and leaves	–
29	*Tecomaria capensis*	branches and leaves	–
30	*Baphicacanthus cusia*	whole	13.4
31	*Alpinia zerumbet*	whole	29.8
32	*Aspidistra elatior*	whole	13.1
33	*Crinum asiaticum*	whole	33.4
34	*Arenga pinnata*	branches and leaves	–
35	*Lophatherum gracile*	whole	–
36	*Excoecaria cochinchinensis*	branches and leaves	28.8
37	*Campsis grandiflora*	branches and leaves	30.4
38	*Wisteria sinesis*	branches and leaves	28.8
39	*Ficus pumila*	whole	15.5
40	*Canarium album*	branches and leaves	33.8
41	*Euonymus japonica*	branches and leaves	52.8
42	*Mimosa pudica*	whole	48.2
43	*Parthenocissus heterophylla*	whole	–
44	*Osmanthus fragrans*	branches and leaves	22.8
45	*Ophiopogon japonicus*	whole	53.6
46	*Saxifraga stolonifera*	whole	11.9
47	*Polygonum multiflorum*	rhizome	–
48	*Podocarpus nagi*	branches and leaves	48.4
49	*Camellia japonica*	branches and leaves	–
50	*Reineckea carnea*	whole	–

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.

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, 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 h. The free and bound ligands were separated by Whatman GF/C glass fiber filters. The radioactivity was measured by scintillation counter. The difference between total radioactivity 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:

$$\% \text{ Inhibition} = \frac{S_c - S_s}{Sc} \times 100 = \frac{(T_c - N_c) - (T_s - N_s)}{T_c - T_s} \times 100$$

*S_c = specific binding of control
S_s = specific binding of sample
T_c = total binding of control
T_s = total binding of sample
N_c = nonspecific binding of sample
N_s = nonspecific binding of control

Results and Discussion

Our study was conducted to evaluate the inhibitory potential of Chinese medicinal plants on the platelet-activating factor (PAF) receptor binding to rabbit platelet. Eighty medicinal plants which have been used traditionally to treat inflammation, allergy, rheumatism and so on were collected and evaluated

Table 1. Inhibitory effects of Chinese medicinal plants on the platelet-activating factor (PAF) receptor binding (continued)

No.	Species	Part used	% Inhibition[a]
51	*Wikstroemia indica*	whole	–
52	*Illicium verum*	branches and leaves	5.2
53	*Pinus massoniana*	branches and leaves	7.8
54	*Chirita eburnean*	whole	8.6
55	*Lycorjs aurea*	whole	9.2
56	*Pinus yunnanensis*	branches and leaves	–
57	*Aglaonema modestum*	whole	–
58	*Gleditsia sinensis*	pod	64.8
59	*Iris tectorum*	whole	–
60	*Ilex cornuta*	branches and leaves	–
61	*Rosa chinensis*	branches and leaves	0.9
62	*Bougainvillea glabra*	branches and leaves	–
63	*Cinnamomum burmanni*	branches and leaves	14.0
64	*Cinnamomum camphora*	branches and leaves	31.2
65	*Gossampinus malabarica*	branches and leaves	11.6
66	*Daucus carota*	rhizome	27.3
67	*Pseudolarix amabilis*	branches and leaves	–
68	*Woodwardia japonica*	whole	4.8
69	*Cycas revoluta*	leaves	32.6
70	*Homalocladium platycladum*	whole	6.8
71	*Acer palmatum*	branches and leaves	–
72	*Clausena lansium*	branches and leaves	64.0
73	*Rhododendron simsii*	branches and leaves	–
74	*Paliurus ramosissimus*	branches and leaves	–
75	*Canna indica*	whole	24.4
76	*Verbena officinalis*	whole	–
77	*Nerium indicum*	branches and leaves	–
78	*Oxalis corymbosa*	whole	31.5
79	*Wedelia chinensis*	whole	23.3
80	*Agave sisalana*	leaves	53.0

[a]concentration: methanolic extract 200 μg/ml; '–' means no activity.

Table 2. Inhibitory potentials of $CHCl_3$, BuOH, and H_2O fractions of methanol extracts of Chinese medicinal plants on the PAF receptor binding.

Species	Part used	% Inhibition of each frac.[a]		
		$CHCl_3$	BuOH	H_2O
Cratoxylon ligustrinum	branches and leaves	73.6	37.9	42.8
Kalimeris indica	whole	81.3	28.4	0.2
Euonymus japonica	branches and leaves	74.7	20.7	–
Ophiopogon japonicus	whole	53.7	4.4	30.4
Gleditsia sinensis	pod	78.5	63.3	7.8
Clausena lansium	branches and leaves	27.6	30.3	77.6
Agave sisalana	leaves	55.0	–	–

[a]Final concentration: 200 μg/ml; – means no activity.

Table 3. Inhibitory potentials of subfractions of $CHCl_3$ partitions of *Kalimeris indica* on PAF receptor binding

Frac.	F1	F2	F3	F4	F5			F6	F7
% Inhibition[a]	–	–	31.9	18.0	68.8			59.6	57.3
Subfrac.					F5-1	F5-2	F5-3		
% Inhibition[a]					45.9	93.6	–		

[a]Final concentration: 100 μg/ml; '–' means no activity.

for their inhibitory activities on platelet-activating factor (PAF) receptor binding. The preliminary study results were summarized in Table 1. The extracts of seven plants, *Cratoxylon ligustrinum*, *Kalimeris indica*, *Euonymus japonica*, *Ophiopogon japonicus*, *Gleditsia sinensis*, *Clausena lansium*, *Agave sisalana* exhibited inhibitory effects of more than 50% at a concentration of 200 μg/ml.

The methanol extracts of these seven plants were partitioned into $CHCl_3$, BuOH and H_2O fractions successively and the partitions were tested for their inhibitory effects. As shown in Table 2, the chloroform fraction of *Kalimeris indica*, *Euonymus japonica*, *Cratoxylon ligustrinum* and *Gleditsia sinensis* shows more potent inhibitory activities than their corresponding methanol extracts. Especially, the chloroform partition of *Kalimeris indica* showed 81.3% inhibitory activity at a concentration of 200 μg/ml. *Kalimeris indica* is a folk medicine used to treat inflammation related diseases such as hepatitis in China (Jiangsu, 1978) and its chemical constituents have not been much investigated. The chloroform partition of *Kalimeris indica* was therefore subjected to silica gel column chromatography eluted with CH_2Cl_2, CH_2Cl_2:MeOH (15:1~1:1) and MeOH gradually to afford seven fractions. Amongst them, the fifth fraction exhibited potent inhibitory activity (68.8% inhibition at a concentration of 100 μg/ml) as shown in Table 3. This fraction was further fractioned by column chromatography using CH_2Cl_2:MeOH:H_2O = 10:5:1 as eluent to give three subfractions. Strong active subfraction was found in Subfraction F5-1 with 93.6% inhibitory activity at a concentration of 100 μg/ml. Therefore, further studies to elucidate the active constituents of this fraction is strongly encouraged.

In conclusion, our preliminary screening study has found several plants which exhibited potent PAF receptor binding inhibitory activity. The characterization of pure active compounds responsible for their activity remained to be further investigated. Novel PAF antagonists are expected to be isolated from these medicinal plants in future study.

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