

Platelet Activating Factor Antagonist Activity of Ginsenosides

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Ginseng saponins and their degradation products have been screened for antagonist activity towards [³H]PAF (platelet activating factor) in washed rabbit platelet receptor binding studies. 20(S)- and Δ^{20} -ginsenosides Rg₃, protopanaxadiol-type saponins, were found to be relatively potent PAF antagonists (IC₅₀=4.9×10⁻⁵ M and 9.2×10⁻⁵ M, respectively).

Key words *Panax ginseng*; PAF antagonistic activity; 20(S)-ginsenoside Rg₃; Δ^{20} -ginsenoside Rg₃

Platelet-activating factor (PAF), identified as 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine,¹⁾ is a biologically active phospholipid originally described as a fluid-phase mediator released after antigenic challenge of sensitized rabbit basophils.²⁾ PAF is active at nanomolar concentrations and exhibits a myriad of physiological and pathological roles, as a hypotensive, increasing vascular permeability, acute inflammation, asthma, cardiac anaphylaxis, thrombosis, gastrointestinal ulceration, endotoxin shock, allergic skin disease and transplanted organ rejection.³⁻⁵⁾ In order to identify PAF receptor antagonists, we screened many kinds of traditional medicines using a PAF receptor binding assay, and PAF receptor binding antagonist activity was found in two saponins from the roots of *Panax ginseng*. We now report the effect of ginsenosides on [³H]PAF binding inhibition activity, and this is the first demonstration that ginsenosides have PAF receptor antagonist activity.

MATERIALS AND METHODS

Plant Materials, Isolation and Preparation of Saponins and Degradation Products The red ginseng used was provided by Korea Ginseng and Tobacco Research Institute. Isolation and preparation of saponins and degradation products were as in the previous study.⁶⁻⁸⁾

Preparation of Reagent Solutions and Buffers ACD (acid-citric acid-dextrose) solution was used as an anticoagulant. Bovine serum albumin (BSA) and ginkgolide B were from Sigma Co. (St. Louis, U.S.A.). Tris-BSA Buffer (10 mM Tris, 10 mM MgCl₂, 30 mM KCl, 1 mM EGTA, 0.1% glucose, 0.25% BSA, pH 7.0) was used for washing platelets, preparing platelet suspensions, dilution of samples and washing filters. Radiolabelled PAF with a specific activity of 142 Ci/mmol and unlabelled PAF were purchased from Amersham (Little Chalfont, U.K.) and Sigma Co., respectively. For the scintillation fluid, Lumagel[†]-safe was purchased from Lumac*LSC B.V. Co. (Olen, Belgium).

Preparation of Samples for PAF Receptor Binding Assay Each sample was dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer (final concentration of DMSO, 0.8%) and 0.8% DMSO in buffer was used as a control.

Preparation of Washed Rabbit Platelet Suspension Five volumes rabbit blood collected by heart puncture was

added to 1 volume ACD solution. The blood was centrifuged at 270 *g* for 10 min, and the top, platelet-rich plasma (PRP) was carefully removed. PRP was centrifuged at 1250 *g* for 10 min, the platelets were then washed by centrifugation in buffer. The final platelet concentration was adjusted to 2×10⁸ cells/ml buffer with a hematocytometer (Brand 717810, Germany).

Inhibition of [³H]PAF Binding to Washed Rabbit Platelets Binding of [³H]PAF to rabbit platelets was carried out according to the methods of Valone⁹⁾ and Yang¹⁰⁾ with some modifications. The reaction mixture consisted of 100 μ l platelet suspension, 90 μ l [³H]PAF (0.9 nM, 70000 dpm), with or without unlabelled PAF (500-fold radioactive form), and 60 μ l sample or control solution. The reaction mixture was incubated at room temperature for 30 min. Free PAF was separated from bound PAF by filtration of the reaction mixture using the MultiScreenTM Filtration System (Millipore Co., U.S.A.). The filters were rapidly washed with ice-cold buffer and then dried and placed into vials containing 3 ml scintillation fluid. Radioactivity was then measured in a liquid scintillation spectrophotometer (Beckman LS6000TA, U.S.A.). The difference between total radioactivity of bound [³H]PAF in the absence and presence of excess unlabelled PAF is defined as the specific binding of the radiolabeled ligand. In a set of experiments, [³H]PAF was incubated with different concentrations of PAF receptor antagonist and the effect of the antagonist on the specific binding was expressed as the percentage inhibition of the control. The IC₅₀ value was defined as the final concentration of the inhibitor required to block 50% of the specific [³H]PAF binding to rabbit platelet receptors. The results of the assay are expressed as the mean of 3 distinct experiments.

RESULTS AND DISCUSSION

The total and specific activities of the PAF receptor binding antagonist activity of ginseng saponins and sapogenin derivatives were assayed. Unfortunately, most of them (ginsenosides Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, 20(R)-Rg₂, 20(S)-Rg₂, 20(R)-Rg₃, 20(S)-Rh₁, 20(R)-Rh₂, 20(S)-Rh₂, Ro, 20(R)-panaxadiol, 20(R)-panaxatriol, 20(R)-protopanaxadiol, 20(S)-protopanaxadiol) had no activity (IC₅₀>200 μ M), so it was difficult to evaluate any structure activity relationship. Only 20(S)-ginsenoside Rg₃ and Δ^{20} -ginsenoside Rg₃ showed

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Table 1. Inhibition of PAF Binding to Washed Rabbit Platelets

Compound	Concentration (μM)	Inhibition (%)	IC_{50}
20(S)-Ginsenoside Rg_3	200	84.4	$4.9 \times 10^{-5} \text{M}$
	100	56.3	
	25	40.3	
	12.5	20.0	
Δ^{20} -Ginsenoside Rg_3	160	73.0	$9.2 \times 10^{-5} \text{M}$
	80	48.2	
	40	29.3	
	20	24.4	
Ginkgolide B			$1.9 \times 10^{-7} \text{M}$

PAF receptor binding antagonist activity. The data are listed in Table 1 and, for comparison, the IC_{50} value of ginkgolide B, a well known potent PAF antagonist from *Ginkgo biloba* leaves, is also shown. The IC_{50} value of 20(S)-ginsenoside Rg_3 was $4.9 \times 10^{-5} \text{M}$ and that of Δ^{20} -ginsenoside Rg_3 was $9.2 \times 10^{-5} \text{M}$, less potent than ginkgolide B.

The PAF antagonists from natural products could be classified as terpenes, lignans, and gliotoxin and related compounds by their skeletons.³⁾ However, ginsenosides, triterpenoid saponins, are different from other reported PAF antagonists. 20(S)-Ginsenoside Rg_3 and Δ^{20} -ginsenoside Rg_3 are thus a new type of PAF antagonist.

Lee *et al.* reported that ginsenoside Rg_3 had an inhibitory effect on thrombin- and collagen-induced aggregation of washed platelets, which was dose-dependent manner.¹¹⁾ Park *et al.* also reported that 20(S)-ginsenoside Rg_3 had the most potent inhibitory activity on multidrug resistance.⁶⁾ Brekham *et al.* suggested that ginseng glycosides had an adaptogenic

activity, which increased non-specific resistance against various stimulant caused diseases.¹²⁾

We suggest that 20(S)-ginsenoside Rg_3 and Δ^{20} -ginsenoside Rg_3 may be responsible for the antiinflammatory effect of *P. ginseng*, especially red ginseng, besides the known effects and our findings have potentially important implications for the therapeutic use of these compounds in inflammatory diseases.

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