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SHORT COMMUNICATION

Antiplatelet effects of acidamides isolated from the fruits of *Piper longum* L.

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Abstract

The inhibitory effects of four acidamides, piperine, pipernonaline, piperoctadecalidine, and piperlongumine, isolated from the fruits of *Piper longum* L. on washed rabbit platelet aggregation were examined. All of the four tested acidamides showed dose-dependent inhibitory activities on washed rabbit platelet aggregation induced by collagen, arachidonic acid (AA), and platelet-activating factor (PAF), except for that induced by thrombin. Piperlongumine, in particular, showed stronger inhibitory effects than other acidamides to rabbit platelet aggregation induced by collagen, AA and PAF.

Keywords: Antiplatelet activity; Piperlongumine; *Piper longum* L.

Introduction

Piper species are distributed widely in the tropical and subtropical regions of the world, have multiple applications in different folk medicines, including the Indian Ayurvedic system, and have been reported to possess numerous biological activities (Stöhr et al., 2001; Shoji et al., 1986; Lee, 2000; Rege et al., 1999; Tripathi et al., 1999). In our previous studies, we isolated four acidamides—piperine, pipernonaline, piperoctadecalidine, and piperlongumine—from the fruits of *Piper longum*. Pipernonaline displayed potent mosquito larvicidal and antifungal activities against phytopathogenic fungi (Lee, 2000; Lee et al., 2001). Piperoctadecalidine also exhibited a broad spectrum of insecticidal activity against five agricultural insect pests (Park et al., 2002). In this study, the inhibitory effects of four acidamides (Fig. 1), piperine (1), pipernonaline (2), piperoctadecalidine (3), and piperlongumine (4), on washed rabbit platelet aggregation *in vitro* were investigated.

Materials and methods

Piperoctadecalidine was available from our previous work (Park et al., 2002). Mass spectra of piperoctadecalidine were determined using a JEOL JMS-DX30 spectrometer and confirmed by comparison to spectra published previously (Ahn et al., 1992). Pipernonaline,

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piperine, and piperlongumine were also isolated from dried fruits of *P. longum* as reported previously (Lee, 2000; Lee et al., 2001).

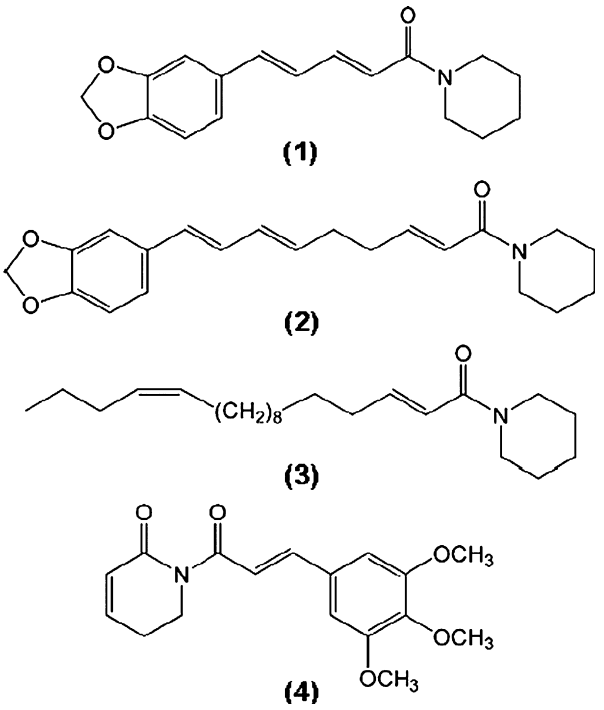

Fig. 1. Acidamides isolated from fruits of *Piper longum* L.

Rabbit (male) blood was collected from the ear aorta with a one-tenth volume of 1% EDTA and centrifuged for 10 min at 230_g. Platelet suspension was prepared from this EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously (Rho et al., 1996). The platelet number was counted using a Coulter Counter (Coulter Electronics, Hialeah, FL, USA) and adjusted to a concentration of 3×10^8 platelets/ml. Platelet aggregation was measured using an aggregometer (470-vs, Chrono-log Co., PA, USA) as described previously (Rho et al., 1996). Briefly, washed platelet suspension (WPS) was incubated at 37 °C for 3 min with DMSO (0.5%, control) or various concentrations of tested compounds for 3 min in the presence of 1 mM $CaCl_2$ in the aggregometer, and platelet aggregation was then induced by addition of collagen (2 μg/ml), arachidonic acid (AA) (100 μM), platelet-activating factor (PAF) (10 nM), or thrombin (0.1 unit/ml). The resulting aggregation, measured as the change in light transmission, was recorded for 10 min. The inhibition rate was obtained from the maximal aggregation induced by the respective agonist at the concentration using the equation inhibition rate = (maximal aggregation rate (MAR) of vehicle-treated WPS − MAR of sample-treated WPS/MAR of vehicle-treated WPS) × 100. Acetylsalicylic acid (ASA, aspirin) was used as a positive control (Kim et al., 1999). Student's *t*-test was used to assess the

Table 1. Inhibitory effects of the acidamides isolated from *Piper longum* fruits on washed rabbit platelet aggregation induced by collagen, AA, PAF, and thrombin

	Conc. (μM)	Aggregation (%)			
		Collagen (2 μg/ml)	AA (100 μM)	PAF (10 nM)	Thrombin (0.1 unit/ml)
Control		72.7 ± 2.9	69.9 ± 2.9	69.3 ± 1.8	80.2 ± 1.3
Piperine	300	6.2 ± 0.3**	2.4 ± 0.4**	1.2 ± 1.1**	75.6 ± 1.5
	150	49.3 ± 1.1*	32.1 ± 3.5*	16.4 ± 2.5**	
	30	71.1 ± 2.5*	55.3 ± 2.1*	59.2 ± 3.2*	
Pipernonaline	300	3.2 ± 0.3**	2.6 ± 0.3 **	3.4 ± 0.2**	76.8 ± 2.1
	150	12.9 ± 0.6*	41.6 ± 3.1*	30.7 ± 0.7*	
	30	79.2 ± 3.1**	67.4 ± 2.8*	57.2 ± 2.3*	
Piperoctadecalidine	300	14.4 ± 2.1*	29.6 ± 0.8*	21.5 ± 3.8**	76.5.0 ± 2.5
	150	49.0 ± 1.5*	54.2 ± 1.6*	52.7 ± 1.5*	
	30	71.2 ± 2.6*	68.8 ± 2.7*	67.6 ± 6.1*	
Piperlongumine	300	0.0 ± 0.0**	0.0 ± 0.0**	0.0 ± 0.0**	61.3 ± 0.9
	150	0.0 ± 0.0**	16.5 ± 2.1**	0.0 ± 0.0**	
	30	36.5 ± 3.9**	61.5 ± 1.7*	52.0 ± 2.4*	
	10	58.2 ± 2.4*	70.1 ± 2.4	68.7 ± 2.1	
Acetylsalicylic acid (aspirin)	300	68.5 ± 3.4	0.0 ± 0.0**	68.2 ± 1.5	81.1 ± 0.7
	150	73.1 ± 2.6	17.5 ± 2.4**	69.1 ± 2.1	
	30		37.8 ± 3.7*		

Washed rabbit platelets were preincubated with DMSO (0.5% control) or each compound at 37 °C for 3 min in the presence of 1 mM $CaCl_2$, and then the inducer was added. Acetylsalicylic acid was used as a positive control. Values are means ± SEM.
*$p < 0.05$, **$p < 0.01$ as compared with the respective control.

significance of differences between the tested compounds and control.

All of the tested four acidamides showed dose-dependent inhibitory activities on platelet aggregation induced by collagen, AA, and PAF, except for that induced by thrombin (Table 1). Piperlongumine had the most potent antiplatelet effect. Piperlongumine inhibited platelet aggregation induced by collagen with inhibition values of 100, 100, and 49.8, and 19.9% inhibitory effects at 300, 150, 30, and 10 μM, respectively. In a test with AA, piperlongumine at 300, 150, and 30 μM exhibited 100%, 76.4%, and 12% inhibitory effects, respectively. Furthermore, piperlongumine at 300, 150, and 30 μM inhibited platelet aggregation induced by PAF with inhibition values of 100%, 100%, and 29.9%, respectively.

Aspirin has been used as a positive reference control due to the potency of its antiplatelet activity on AA-induced platelet aggregation and the fact that it inhibits only AA-induced platelet aggregation (Table 1). Exogenous AA is converted into prostaglandin endoperoxides by cytosolic cyclooxygenase (COX) and then converted by thromboxane synthase to thromboxane A_2, a potent inducer of platelet aggregation (Vargaftig et al., 1981). The mode of antiplatelet activity of aspirin is due to its inhibition on COX (Vane, 1971). In our results, piperlongumine has showed stronger antiplatelet activity against collagen-induced platelet aggregation rather than AA or PAF, suggesting that the antiplatelet mechanism of piperlongumine may be, at least partly, different from aspirin.

The comparison of these acidamides' structures shows that a pyridone and pyridine moiety might be required for antiplatelet activity. A trimethoxybenzene moiety also seems to contribute to a more potent inhibitory effect on collagen. Similar studies of *N*-methoxycarbonyl aporphines isolated from *Rollinia mucosa* on antiplatelet activity have shown significant inhibition of collagen-, AA-, or PAF-induced platelet aggregation (Kuo et al., 2001) according to the position of the methoxy functional groups. Therefore, the trimethoxybenzene moiety of piperlongumine may play an important role in the activity against collagen-induced platelet aggregation.

In the present study, we suggest that piperlongumine isolated from *P. longum* may be useful as a lead compound and new agent for the prevention or treatment of thrombosis. The inhibitory mechanism and other pharmacological actions of piperlongumine are also currently under investigation.

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