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Anti-inflammatory Effect of *Patrinia villosa* Extract on Proteinase-activated Receptor-2 Mediated Paw Edema

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Proteinase 활성수용체-2 유발 흰쥐 발바닥 부종에 미치는 패장근 물추출물의 항염증 효과

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ABSTRACT : The root of *Patrinia villosa* Jussieu (Valerianaceae) has long been used for treatment of infectious diseases in Korea. In this study, the anti-inflammatory effect of the *Patrinia villosa* root water extract (PVWX) was investigated in proteinase-activated receptor-2 (PAR2)-mediated rat paw edema. Paw edema was induced by injection of trypsin or *trans*-cinnamoyl-LIGRLO-NH_2 (*tc*-NH_2) into hindpaw of rats. PVWX (10, 50, 100 and 200 mg/kg) was orally administered 1 h before the induction of inflammation. At doses of 50, 100 and 200 mg/kg, PVWX showed significant inhibition on both change in paw volume and vascular permeability. PVWX (100 mg/kg) significantly inhibited PAR2 agonists-induced myeloperoxidase (MPO) activity in paw tissue. These results indicate that PVWX has an anti-inflammatory action in PAR2-mediated paw edema.

Key words : *Patrinia villosa*, anti-inflammatory, trypsin, *tc*-NH_2, paw edema

INTRODUCTION

Recently a new family of G-protein coupled, seven trans-membrane domain receptors has been described that are activated by proteolytic cleavage of their extracellular N-terminus (Vu *et al.*, 1991; Hollenberg *et al.*, 1996; Ishihara *et al.*, 1997). Four members of this family have been cloned. Proteinase-activated receptor PAR1, PAR3 and PAR4 can be activated by thrombin (Kahn *et al.*, 1998; Coughlin, 1999) and PAR2 and PAR4 can be activated by trypsin, and mast cell tryptase also activates PAR2 (Corvera *et al.*, 1997; Kong *et al.*, 1997; Molino *et al.*, 1997). Trypsin, mast cell tryptase, or synthetic peptides (SLGRL-NH_2) corresponding to the tethered ligand of PAR2 can activate PAR2 and induce widespread inflammation (Steinhoff *et al.*, 2000). It is believed that PAR2 plays important roles in the process of inflammation, which is caused by increase of vascular permeability (Emilsson *et al.*, 1997), infiltration of neutrophils (Vergnolle *et al.*, 1998) and secretion of proinflammatory cytokines (Hou *et al.*, 1998). Therefore, scavenging one of these phases and inhibition of PAR2 activation are two pharmacological strategies for the treatment of PAR2-mediated inflammation.

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The root of *Patrinia villosa* has long been used for treatment of infectious diseases in East Asian countries. In traditional Chinese therapy, it is supposed that *Patrinia villosa* dispels noxious heat from blood and neutralizes the poisonous effects (Huang, 2000). And *Patrinia villosa* significantly increases blood neutrophil activity and promotes the neutrophil phagocytosis (Hu *et al.*, 1992). In this study, the anti-inflammatory effects of *Patrinia villosa* water extract (PVWX) was investigated in rat paw edema caused by trypsin or *trans*-cinnamoyl-LIGRLO-NH_2 (*tc*-NH_2) (Saifeddine *et al.*, 1998).

MATERIALS AND METHODS

Materials and animals

Trypsin, hexadecyltrimethyl-ammonium bromide (HTAB), *o*-dianisidine was purchased from Sigma (MO, USA). *tc*-NH_2 (corresponding to human PAR2 tethered ligand, M.W. 812.59) was purchased from Santa Cruz Biotech. Inc. (CA. USA).

Experiments were performed on male Sprague-Dawley rats (150~200 g) purchased from Da Mool Science (Daejeon, Korea). Animals received a standard laboratory diet and water *ad libitum* in an air conditioned room under constant temperature (22±2℃) and were kept under a 12 h light-dark cycle.

Plant materials and extract preparations

The roots of *Patrinia villosa* was purchased from the oriental drug store in Jeonju. PVWX was prepared by decoction of the sliced roots of *Patrinia villosa* for about 3 hrs with distilled water (100 g/ℓ). The extract was filtered through a 0.45 ㎛ filter, lyophilized, and kept at 4℃. The dried extract was dissolved in sterile saline before use.

Induction and measurement of Trypsin and *tc*-NH_2-induced paw edema

Rats with free access to water but fasted overnight (18 hrs) were injected intramuscularly with ketamine HCl (30 ㎎/㎏) and xylazine (6 ㎎/㎏). PVWX (10, 50, 100 and 200 ㎎/㎏) or the vehicle (saline solution 0.9% w/v NaCl) was administered by oral route 1 hr before the injection of trypsin (500 ㎮) or *tc*-NH_2 (500 ㎍) into the subplantar area of the hindpaw (n=6 per group). The trypsin or *tc*-NH_2 was dissolved in sterile 0.9% saline and injected in a volume of 100 ㎕. The size of edema was assessed by measuring the volume of the hindpaw immediately before and 1 hr after the intraplantar injection, and is expressed as change of paw volume from the value before intraplantar injection. The paw volume was measured with a plethysmometer (Ugo Basile, Italy).

Measurement of vascular permeability

The changes in vascular permeability following the injection of trypsin or *tc*-NH_2 were evaluated by the Evans blue leakage technique. The rats were orally administered with PVWX (10, 50, 100 and 200 ㎎/㎏) and the vehicle (saline solution 0.9% w/v NaCl). Rats received an intravenous (*i.v*) injection of 2.5 ㎎/㎏ Evans blue in saline, immediately before the intraplantar injection of trypsin or *tc*-NH_2. One hour after the agonists injection, the rats were sacrificed and the paws removed and weighed. Extravasated dye from the paw was measured by method of Katayama *et al.* (1978). Briefly, a piece of the paw containing extravasated dye was soaked overnight in a stoppered glass tube containing 1 ㎖ of 1 N KOH at 37℃. Then, 9 ㎖ of mixed solution of 0.6 N H_3PO_4 and acetone (5:13) was added to the tube. The tube was shaken vigorously for a few seconds and centrifuged at 3,000 rpm for 15 min. Absorbance of supernatant was measured at 620 ㎚ using a spectrophotometer. The concentration of dye was determined from a standard curve of Evans blue. The extravasation of Evans blue was expressed as microgram per paw gram.

The inhibition percentage of paw volume or vascular permeability were calculated using the following equation:

$$\text{Inhibition } (\%) = (A-B)/A \times 100$$

Where A is change of paw volume or vascular permeability in rats without PVWX, and B is change of paw volume or vascular permeability in paw of rats with PVWX.

Myeloperoxidase assay

Oral administration of saline or PVWX was

performed 1 hr prior to subplantar injection of trypsin or *tc*-NH_2 (n= 6 per group). Six hours after agonist injection, paw was weighed and assessed by a modified myeloperoxidase (MPO) assay as previously described (Bradley *et al.*, 1982). This assay was effective in recovering 97% of the added activity, indicating that MPO from neutrophils had accumulated in inflamed tissue (Bradley *et al.*, 1982). In this assay the within-run and between-run was minced and homogenized in KH_2PO_4/K_2HPO_4 buffer (pH 6.0) containing 0.5% HTAB at 0℃ for 45 sec. in motor-driven homogenizer. And then centrifuged at 3,000 rpm for 20 min. at 4℃. For the assay of myeloperoxidase activity, the following reagents were added to wells of a 96-well microtiter plate: 50 ㎕ of supernatant, 50 ㎕ of phospate buffer containing 0.5 % HTAB (pH 6.0), 50 ㎕ of *o*-dianisidine (0.68 ㎎/㎖ in didtilled water), and to start the reaction 50 ㎕ of freshly prepared 0.003 % hydrogen peroxide. The change in absorbance was measured spectrophotometrically at 450 ㎚. The inhibition percentage of myeloperoxidase activity was calculated using the following equation:

$$\text{Inhibition}(\%) = (A-B)/A \times 100$$

Where A is myeloperoxidase activity in paw of rats without PVWX, and B is myoloperoxidase activity in paw of rats with PVWX.

Statistical analysis

Values are expressed as mean±S.E. Statistical significance was determined using the Student's *t*-test, value with $p<0.01$ was considered significant.

RESULTS

PVWX inhibits PAR2-mediated hind paw edema

Subplantar injection of the PAR2 agonists, trypsin or *tc*-NH_2, caused edema, the volume of which was significantly greater than that of the saline alone 1 hr after PAR2 agonist injection (Table 1). In the trypsin-induced edema, PVWX showed significant inhibition at dose of 50 ㎎/㎏ (39.6%), 100 ㎎/㎏ (70.8%) and 200 ㎎/㎏ (72.9%). In the *tc*-NH_2-induced edema, PVWX showed also significant inhibition at dose of 50 ㎎/㎏ (37.5%), 100 ㎎/㎏ (65.0%) and 200 ㎎/㎏ (65.0%). The inhibition percentage at dose of 100 ㎎/㎏ on paw edema showed a maximal effect.

Table 1. Effects of PVWX on trypsin or *tc*-NH_2-induced paw edema in the rats.

Agonist	Treatment	Dose (㎎/㎏ *p.o.*)	Change of paw edema (㎖)	% inhibition
Trypsin (500 ㎪)	Saline	-	0.48±0.04	-
	PVWX	10	0.45±0.14	6.3
		50	0.29±0.05*	39.6*
		100	0.14±0.03*	70.8*
		200	0.13±0.01*	72.9*
tc-NH_2 (500 ㎍)	Saline	-	0.40±0.11	-
	PVWX	10	0.37±0.07	7.5
		50	0.25±0.06	37.5*
		100	0.14±0.07*	65.0*
		200	0.14±0.04*	65.0*

Data show the mean±SE from six rats. *p<0.01 compared to the saline group.

PVWX inhibits vascular permeability

Vascular permeability of Evans blue was markedly enhanced by trypsin and *tc*-NH_2. PVWX (50, 100 and 200 ㎎/㎏) significantly inhibited the Evans blue extravasations to the paw tissue (Table 2). At dose of 100 ㎎/㎏, PVWX showed 65.8% inhibition in the trypsin-induced permeability and 54.6% inhibition in the *tc*-NH_2-induced permeability, showing a maximal effect in this dose.

Table 2. Effects of PVWX on trypsin or *tc*-NH_2-induced vascular permeability in the paw of rats.

Agonist	Treatment	Dose (mg/kg *p.o.*)	Amount of drug (μg/g paw)	% inhibition
Trypsin	Saline	-	91.30±6.71	-
(500 pmol)	PVWX	10	74.22±7.01	18.7
		50	57.11±5.17*	37.4*
		100	31.24±4.22*	65.8*
		200	32.01±3.58*	64.9*
tc-NH_2	Saline	-	70.27±5.11	-
(500 μg)	PVWX	10	61.48±3.91	12.5
		50	45.61±2.98	35.1*
		100	31.88±4.01*	54.6*
		200	30.79±4.71*	56.2*

Data show the mean±SE from six rats. *p<0.01 compared to the saline group.

PVWX inhibits myeloperoxidase activity

Subplantar injection of trypsin or *tc*-NH_2 into hind paw of rats led to an extensive cellular infiltration 6 hrs after agonist injection. PVWX (100 mg/kg) significantly inhibited the MPO activity of the paw tissue (Table 3). PVWX (100 mg/kg) showed 64.5% inhibition in the trypsin-induced edema and 58.7% inhibition in the *tc*-NH_2-induced edema.

Table 3. Effects of PVWX on trypsin or *tc*-NH_2-induced MPO activity in the paw of rats.

Agonist	Treatment	Dose (mg/kg *p.o.*)	MPO activity (unit/g paw)	% inhibition
Trypsin	Saline	-	5.61±0.42	-
(500 pmol)	PVWX	100	1.99±0.15*	64.5*
tc-NH_2	Saline	-	4.41±0.38	-
(500 μg)	PVWX	100	1.82±0.24*	58.7*

Data show the mean±SE from six rats. *p<0.01 compared to the saline group.

DISCUSSION

This study showed that PVWX significantly reduced the trpysin or *tc*-NH_2-induced change of paw edema, vascular permeability and neutrophil infiltration at doses of 50, 100 and 200 mg/kg. Injection of PAR2 agonist such as trypsin or *tc*-NH_2 into the rat paw resulted not only in an increase in vascular permeability, as reflected by the edema, but also in the marked infiltration of granulocytes, another hallmark of inflammatory reactions (Kawabata *et al.*, 1998; Vergnolle *et al.*, 1999). These inflammatory reactions are suitable model for evaluation of the anti-inflammatory agents. PAR2 is expressed not only on neutrophils (Howells *et al.*, 1997) and eosinophil but also on mast cells (Miike *et al.*, 2001). Kawabata *et al.* (1998) demonstrated a rapid (15 min.) mast cell-dependent increase in tissue permeability (Evans blue extravasation) caused by the administration of the PAR2 activating peptide (AP), SLIGRL-NH_2. In contrast, Vergnolle *et al.* (1999) demonstrated that the inflammatory response induced by *tc*-NH_2, is largely independent of mast cell degranulation. These findings suggest that PAR2 presents in tissue components other than mast cell such as neutrophil and eosinophil as well as endothelial cells (Nystedt *et al.*, 1996) may represent one of the principle targets for trypsin or *tc*-NH_2 in

the paw, in terms of causing increased tissue permeability and edema. Vergnolle *et al.* (1999) reported that the paw edema induced by *tc*-NH_2 does not involve either prostaglandins or nitric oxide. The effectiveness of PVWX might be explained by hypothesis that some active constituent of PVWX may act as an inhibitor for tissue permeability.

The results presented in this study should be taken as a base for further investigation on the action of individual constituents of the extract. Due to the significant anti-inflammatory activity exhibited in this model, PVWX has the potential to be used as a cure in inflammatory conditions.

적 요

패장 (*Patrinia villosa* Jussieu, Valerianaceae)의 뿌리는 한국에서 오래전부터 감염성 질환의 치료에 사용되어 왔다. 본 논문에서는 패장근의 물 추출물 엑스 (PVWX)가 proteinase 활성수용체-2 (PAR2)에 의하여 유발된 흰쥐 발바닥 부종에 대한 항염증효과를 연구하였다. 발바닥 부종은 trypsin이나 *trans*-cinnamoyl-LIGRLO-NH_2 (*tc*-NH_2)를 쥐의 발바닥에 주사하여 유발시켰다. PVWX (10, 50, 100 and 200 ㎎/㎏)는 부종유발 1시간 전에 경구로 투여하였다. 50, 100 및 200 ㎎/㎏의 PVWX 투여시 부종의 부피와 혈관투과성의 변화에 유의성 있는 억제를 나타냈다. PVWX (100 ㎎/㎏)은 발바닥 조직에서 PAR2 작동약으로 유발된 myeloperoxidase (MPO)활성을 유의성 있게 억제하였다. 이러한 결과들은 PVWX가 PAR2로 유발된 쥐의 발바닥 부종에서 항염증효과가 있음을 보여준다.

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