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Antiinflammatory Action of Phytolaccosides

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Phytolaccosides의 消炎作用

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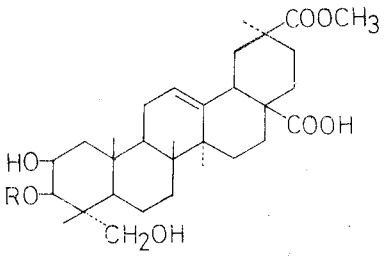
Antiinflammatory action of phytolaccosides B and E isolated from the roots of *Phytolacca americana* was investigated and compared with that of aescin. Phytolaccoside B, when it was administered intravenously, exerted a potent inhibitory effect on the transudation caused by absorptive sponge pellet implanted s.c. in rats and on carrageenin induced edema in rats. Phytolaccoside E showed a weaker action than Phytolaccoside B did. Hemolytic potency of the saponins was decreased in the order of aescin, phytolaccosides B and E showing a same tendency as in antiinflammatory action. The experimental result suggests that the mode of action of phytolaccosides is the same as that of aescin.

Introduction

The roots of *Phytolacca* plants have long been used as an indigenous medicine in the treatment of edema and rheumatism from ancient times.

In the previous papers, it has been reported that saponin mixture from the roots of *Phytolacca americana* named phytolaccoside has an antiinflammatory action against an acute edema as detected by paw edema method[1,2] and the structures of five saponins were determined by Woo *et al*[3~6].

The present experiment, therefore, was undertaken to investigate the antiinflammatory properties of phytolaccosides B and E which are main saponins and to clarify the relationship between structure and action of the saponins.



	R
Phytolaccagenin	H
Phytolaccoside B	β-Xyl
Phytolaccoside E	β-Glu(1→4)β-Xyl

Materials and Methods

Animals Male rats of Sprague Dawley strain weighing 170-200g and albino mice weighing 20±2g were used. Male rabbits weighing 2.5kg were also used. The animals were fed lab. chows and tap water *ad. lib.* During experiments, the animals were given tap water only.

Materials Phytolaccosides B and E were isolated and crystallized according to the procedure described in a previous paper[3]. Sodium salt of phytolaccosides was prepared as followings: phytolaccosides were reacted with a stoichiometric amounts of Na_2CO_3 in ethanol. The reaction mixture was evaporated to dryness at 50° under reduced pressure. The residue thus obtained was dissolved in 0.9% NaCl solution and used for the experiments. γ-Aescin was kindly supplied from Bukwang Pharm. Co., Seoul.

Acute toxicity The acute toxicity of the saponins was measured by determining LD_{50} for mice and rats. The animals were administered with the saponins intravenously and were observed for any change in behavior and 72hr later to assess mortality. LD_{50} was calculated by the method of Behrens-Kaerber[7] and up and down method.

Hemolysis test The method of Fujita *et al*[8]. was employed. One ml aliquot of rabbit blood obtained by cutting carotid artery was mixed with 0.1 ml of 3.6% sodium citrate solution and then diluted up to 50 times with the addition of isotonic phosphate buffer solution (pH 7.4) to make a final 2% blood suspension. To 1 ml of this blood suspension 1 ml each of the dilute saponin solution of various concentrations was added, thoroughly mixed and stood at room temperature for 5 hr. The hemolytic potency was determined by the dilution ratio of the saponins in the reaction mixture which caused a complete hemolysis. The hemolytic index was calculated by the following equation:

$$\text{H. I.} = \frac{V}{\frac{p}{100} \times S}$$

in which V=total volume in the test tube
P=saponin % in the sample solution
S=the amount of the sample used.

Exudative activity test The method of Vogel *et al*[9]. was employed. Cylindrical sponge pellets were made by punching the polyurethane sponge of about 1 cm in height placed horizontally with a cork borer of 0.5 mm in diameter. The sponge pellets thus made were defatted by washing with ether, sterilized by heating for 16 hr at 105° and were used for experiment.

The sterilized sponge pellets(5.1±0.04 mg) were implanted s.c. through the incision on the back of rats under ether anesthesia.

The implanted sponge pellets absorbed exudate rapidly during the first 60 min and thereafter the weight of the exudate increased slowly (Fig. 1). Sixty minutes after the implantation of the sponge pellets, the rats were killed by anesthetizing with ether. The dorsal skin was incised to open and the pellets were removed with a fine pincet without giving any tension. The amount of the exudate absorbed for the first one hour was measured by weighing the pellet wet weight.

Rat paw edema test The method described in a previous paper[2] was used with a modification of the dosage schedule of the saponins. A volume of 0.1 ml of 1% carrageenin in 0.9% NaCl was injected into the subplantar side of right hind paw of the rats. The saponins dissolved in 0.9% NaCl were injected intravenously 16 hr before the injection of the irritant. The volume of the paw was measured plethysmometrically immediately and 3 hr after the carrageenin treatment. Degree of edema was expressed as the volume increased after carrageenin injection.

Results

Acute toxicity The results of the acute toxicity tests were indicated in Table I. It was shown that the acute toxicity of the saponins for mice was higher than that for rats giving approximately 2 times differences in their LD_{50} values. Aescin was the most toxic. During 10–16 hr after the i.v. administration of the saponins the animals were observed weight loss, fatigue and decreased respiration, and death were followed.

Table I. Acute Toxicity of Phytolccosides and Aescin.

Compounds	Species	Route	LD_{50} (mg/kg)
Aescin	Mouse[a)]	i. v.	3.2
	Rat[b)]	i. v.	5.5
Phytolaccoside B	Mouse	i. v.	4.5
	Rat	i. v.	10.8
Phytolaccoside E	Mouse	i. v.	23.6
	Rat	i. v.	42.3

a) Behrens-Kaerber method.
b) Up and down method.
The animals were observed for 72 hours after dosing.

The survived animals were rapidly regained their body weight and recovered from the symptoms.

The hemolytic activity The hemolytic activity of the saponins was summarized in Table II.

The hemolytic activity was expressed as hemolytic indice(crude index). Aescin showed the highest hemolytic potency which was even stronger than that of the reference saponin(S.B. Penick & Co., U.S.A.). The hemolytic index of phytolaccoside B was about one half of that of the reference saponin. Phytolaccoside E showed a relatively low hemolytic potency.

Anti-exudative action Fig. 1 shows the result of time dependent changes in the exudate formation after the s.c. implantation of the sponge pellets. The weight of the exudate was observed to increase rapidly during first 1 hr and then increase slowly thereafter. From this result, the total amount of the exudate formed during 1 hr after the implantation was used as a measure of the exudative reaction.

Table II. Comparison of Hemolytic Indices of Phyto laccosides and Aescin Tested by Using Rabbit Blood in Phosphate Buffer Solution (pH 7.4).

Compounds	Hemolytic index (Crude index)
Saponin(Reference)	40,000
Aescin	66,667
Phytolaccoside B	20,000
Phytolaccoside E	7,143

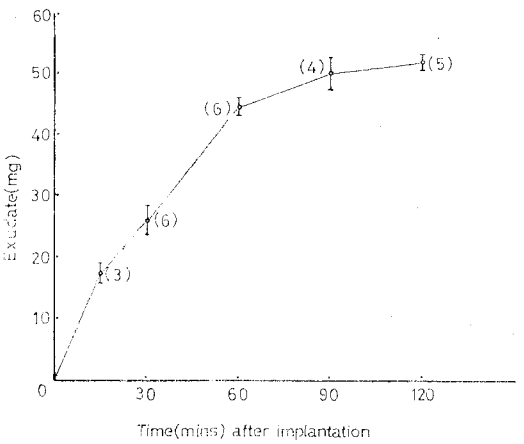


Fig. 1. Time dependent changes of exudate amount after s.c. implantation of absorptive polyurethane sponge pellets.
Vertical lines represent S. E. of the mean. Each point is the mean value of the exudate weight. Figures in parentheses are the number of animals.

Graded doses of phytolaccosides and aescin were administered intravenously sixteen hours prior to the determination of their anti-exudative properties. The extent of local exudation was determined by weighing the wet weight of the sponge implanted. As indicated in Table III, the wet weight of the sponge pellets was dra-

stically decreased by the administration of 2 mg/kg(about 1/2 of LD_{50} value) of aescin. Phytolaccoside B was also shown to be effective at the doses between about 1/4 −3/4 of LD_{50} value, giving linear dose response effects.

Phytolacoside E exhibited a low activity. Only at higher dose employed (3/4 of LD_{50} value), a significant inhibitory effect of the exudative reaction was observed. ED_{50} value of phytolaccoside B was calculated to be 5.8 mg/kg, and its approximate therapeutic index was about a half of the value of aescin.

Table III. Influences of Aescin and Phytolaccosides on the Exudate Formation after s.c. Implantation of an Absorptive Artificial Body.

Compounds	No. of rats	Dose (mg/kg, i.v.)	Exudate		Inhibition (%)	ED_{50} (mg/kg)
			Control	Treated		
Aescin	5	2	45.8±2.8	15.8±3.2[a]	66.0	
	6	1	45.8±7.0	26.3±3.5[b]	42.0	
Phytolaccoside B	6	7.9	45.8±2.8	18.4±3.5[a]	60.0	
	6	5.2	49.8±3.5	27.4±5.0[a]	45.0	5.8
	5	2.6	45.8±7.0	30.6±2.4[c]	28.0	
Phytolaccoside E	6	31.0	45.8±2.8	37.1±2.5[b]	19.0	
	5	20.7	49.8±3.5	43.3±6.5	13.1	
	5	10.3	45.8±7.0	43.7±4.9	4.5	

The materials were injected intraveneously 16 hours before the implantation of the sponge pellets. One hour after the implantation, the sponge pellets were removed and their wet weights were weighed.
a) $P<0.01$ vs. the control, b) $P<0.05$ vs. the control and c) $0.05<P<0.1$ vs. the control.

Anti-edemic action The inhibitory effect of phytolaccosides and aescin on edema induced by carrageenin was tested and their activity was compared at doses of around 1/2 of their LD_{50} values. As seen in Table IV, aescin and phytolaccosides exhibited significant inhibitory effects on acute edema. Aescin was also the most active and the potency of the saponins showed a similar tendency as in anti-exudative action.

Table IV. Effects of Phytolaccosides and α-Aescin on Carrageenin Induced Edema in Rats.

Compounds	Dose (mg/kg, i.v.)	No. of rats	Edema volume (ml)	Inhibition (%)
Control	−	6	0.87±0.09	
Aescin	2	6	0.29±0.09[a]	66.7
Phytolaccoside B	5.2	6	0.34±0.09[b]	60.9
Phytolaccoside E	20.7	6	0.52±0.07[c]	40.2

a) $P<0.01$ vs. the control, b) $P<0.02$ vs. the control and c) $P<0.05$ vs. the control.
Data were expressed as mean± S. E. M. of edema volumes measured at 3 hours after the injection of carrageenin.

Discussion

It is known that the progress of inflammatory reaction is complicated ; primary disorders of capillary permeability, cellular infiltration in inflammatory region and cell proliferation associated with neoformation of connective tissue[10].

Typical antiexudative agents exert their influence especially on the disorder of capillary permeability in the first phase of inflammation.

Aescin, a saponin isolated from horse chestnut has been demonstrated to be a very potent anti-edemic[11~14] and anti-exudative[9] agent rather than an agent which has a direct effect upon connective tissue. The marked dehydration occuring after giving aescin intravenously was considered to be due to major cell membrane permeability changes[14]. In our previous experiments[2], it was also reported that saponin mixture of *Phytolacca* plant possessed a strong anti-edemic property, however, exhibited a significant anti-granulomatous action only at higher dose. Phytolaccosides, therefore, are expected to be more effective to an early phase of the inflammation.

It was demonstrated previously that the anti-edemic and anti-exudative effect of aescin reached a maximum 16 hr after i.v. administration[11]. In order to compare the antiinflammatory efficacy of phytolaccosides with that of aescin, the same dosage schedule as that used in testing the action was employed in the present experiments. The present results clearly indicated that phytolaccosides B and E exerted both anti-edemic and anti-exudative actions (Table III, and IV).

Judging from the experimental results thus far obtained, the mechanisms of antiinflammatory action of phytolaccosides are considered to be the same as those of aescin.

Phytolaccoside B showed much stronger actions than phytolaccoside E did. The acute toxicity and the hemolytic potency of the saponins were decreased in the order of aescin, phytolaccosides B and E. It strongly suggests that toxicity of saponins are related to their hemolytic pontencies.

It is worthy to note that phytolaccoside B which possesses only one sugar molecule(xylose) on 3β-OH position not only gave much stronger hemolytic potencies but also anti-exudative action than phytolaccoside E which bears just one additional glucose molecule.

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