Arch Pharm Res Vol 22, No 4, 401-403, 1999

Anti-Platelet Aggregation Activity of Stilbene Derivatives from *Rheum undulatum*

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(Received April 19, 1999)

In continued studies on cultivated Korean rhubarb rhizomes (*Rheum undulatum*), three known stilbenes (desoxyrhapontigenin, rhapontigenin, piceatannol) have been screened for activity on blood platelet aggregation. Both rhapontigenin and desoxyrhapontigenin exhibited strong inhibition on the aggregation induced by arachidonic acid and collagen. However, piceatannol did not show inhibition. These inhibitory effects may partially contribute to anti-blood stagnancy activity of rhubarb.

Key words : Platelet aggregation, *Rheum undulatum*, Cultivated Korean rhubarb rhizomes, Stilbenes, Desoxyrhapontigenin, Rhapontigenin, Piceatannol

INTRODUCTION

Rhubarb is one of the oldest and best-known chinese and western medicine. In Europe, the rhubarbs are commonly used as a purgative. They have been used as purgative, anticoagulant, antiphlogistic, antipyretic, antijaundice, etc. in chinese traditional herbal therapy. On the other hand, rhubarb has been blended in prescriptions, such as DaeWhangMokTanPiTang (大黃牧丹皮湯) and DoHaekSeungKiTang (桃核承氣湯) for treatment of Ohyul (瘀血) symptom in the Chinese herbal therapy. Regarding pharmacological studies of rhubarb, cathartic activity of sennoside (Miyamoto *et al.*, 1967), decreasing effect on the urea nitrogen levels in blood (Shibutani *et al.*, 1980), effect of adenine-induced renal failure (Yokozawa *et al.*, 1983), antibacterial activity of aloe-emodin (Haruda *et al.*, 1983), analgesic activity of lindleyin (Darias *et al.*, 1987), spasmolytic activity of stilbenes (Chandhari *et al.*, 1983), antitumor activity of stilbenes (Ryu *et al.*, 1994), anti-allergic and anti-inflammatory activity (Kubo *et al.*, 1997), as well as an anticomplementary activity of stilbenes (Oh *et al.*, 1998) have been reported. However, the effect of rhubarb against Ohyul symptoms has not been investigated. Ohyul has been considered to be closely related to blood coagulation, inflammation, and immunity. Consequently, effects of stilbenes from cultivated Korean rhubarb rhizomes (*Rheum undulatum*) on blood platelet aggregation were studied.

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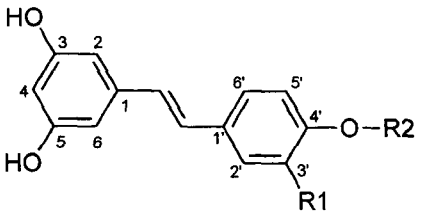
MATERIALS AND METHODS

In previous paper, the isolation of three stilbenes (desoxyrhapontigenin, rhapontigenin, piceatannol) from cultivated Korean rhubarb rhizomes (*Rheum undulatum*) has been reported.

The structures of three stilbenes are shown in Fig. 1. Following materials were used in this study : arachidonic acid (Sigma), and collagen (Chrono-log).

BLOOD PLATELET AGGREGATION TEST

Whole blood samples were collected from the carotid



	R1	R2
Desoxyrhapontigenin	**H**	**CH_3**
Rhapontigenin	**OH**	**CH_3**
Piceatannol	**OH**	**H**

Fig. 1. Structure of stilbene derivatives from *Rheum undulatum*.

Table I. Effects of stilbenes from *Rheum undulatum* and aspirin on arachidonic acid and collagen-induced blood platelet aggregation

Treatment	Conc. (mM)	Arachidonic acid (100 μM)		Conc. (mM)	Collagen (10 μg/ml)	
		Aggregation(%)	Inhibition(%)		Aggregation(%)	Inhibition(%)
Control		58±4.1			61±1.4	
Desoxyrhapontigenin	0.05	58±3.4	0	0.1	51±1.2	16
	0.076	58±4.1	0	0.2	27±3.1*	56
	0.1	0**	100	0.5	8±1.0**	87
	0.2	0**	100	1.0	6±1.0**	90
Rhapontigenin	0.008	56±3.9	3	0.05	48±1.9	21
	0.01	44±3.2	24	0.1	22±1.2**	64
	0.02	0**	100	0.25	12±0.4**	80
	0.05	0**	100	0.5	0**	100
Piceatannol	1.5	52±2.7	10	0.5	52±3.6	15
	3.0	52±3.0	10	1.0	50±2.7	18
Aspirin	0.05	11±3.0**	81	0.01	56±3.0	8
	0.1	5±1.8**	91	0.03	33±3.0*	46

Each value represents the mean±S.E. of 4 experiments. Significantly different from the control, *p<0.05, **p<0.01.

of pentobarbital-anesthetized rabbits. The blood of rabbit (9 ml) and sodium citrate (3.8%, 1 ml) were transferred into a plastic tube, and centrifuged at 1200 r.p.m for 10 min to obtain platelet-rich plasma (PRP). PRP was removed with a screw cap. The remaining blood sample was further centrifuged at 3000 r.p.m for 30 min to give platelet-poor plasma (PPP), which was used as a maximal transmittance standard.

The blood platelet aggregation test was performed according to the method of Born *et al.* Arachidonic acid (100 μM), collagen (10 μg/ml)-induced blood platelet aggregation test were used with PRP prepared from rabbits. 315 μl aliquot of PRP was placed in a test tube and the content was stirred at 1200 r.p.m for 3 min at 37°C, then 5 μl aliquot of a test solution was added. After 3 min, an aggregating agent was added to the reaction mixture. Changes in the light transmittance of the reaction mixture were continuously recorded with a Husm system platelet aggregometer (Chrono-log corporation) and the transmission at the maximal aggregation after the addition of an aggregating agent was recorded. Platelet aggregation was expressed as the percent increase in the transmittance taking the transmittance of a control mixture containing no test solution as zero. Anti-platelet aggregating agent, aspirin, was used as a standard.

RESULTS AND DISCUSSION

Rhubarb has been used in medicines for treatment of the Ohyul symptoms in Chinese medicinal prescription. The Ohyul believed to be caused by blood coagulation, inflammation and immunity. Experimental models against Ohyul symtoms were investigated on blood platelet aggregation. As shown in Table I, desoxyrhapontigenin and rhapontigenin exhibited strong inhibition on the aggregation induced by arachidonic acid and collagen, while piceatannol did not produce inhibitory effect. Rhapontigenin showed stronger inhibitory effect on arachidonic acid and collagen-induced blood platelet aggregation than that of desoxyrhapontigenin. Rhapontigenin and desoxyrhapontigenin showed full inhibition (100%) on the aggregation induced by arachidonic acid at the conc. of 0.02 mM, and 0.1 mM respectively and also almost full inhibition (100, 90%) on the aggregation induced by collagen at the conc. of 0.5 mM, and 1.0 mM respectively. 0.1 mM Aspirin showed potent inhibitory effect. Rhapontigenin and desoxyrhapontigenin showed equal or superior inhibitory effects when compared to aspirin. These results suggested that anti-platelet aggregation activity of stilbenes from *Rheum undulatum* were effected by the presence of methoxyl and free hydroxy groups of the structure. The inhibition activity on platelet aggregation induced by arachidonic acid is considered to be closely related to the arachidonate metabolism. Consequently, these inhibitory effects may partially contribute to anti-blood stagnancy activity of rhubarb. Stilbenes can be considered as pharmaco-constituents of *Rheum undulatum*. Anti-platelet aggregation activity of stilbenes from *Rheum undulatum in vitro* is reported for the first time in this paper. Further study is in progress on the effects of these stilbenes on *in vivo* experimental thrombosis in rats.

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