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Antihaemostatic and antithrombotic effect of some antiplatelet agents isolated from Chinese herbs

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Abstract—Five antiplatelet agents have been isolated from Chinese herbs. Apigenin and magnolol are inhibitors of thromboxane synthesis, while osthole, protopine and norathyriol are inhibitors of phosphoinositide breakdown. Thirty min after intraperitoneal (i.p.) administration of these drugs, tail bleeding time of mice was prolonged markedly in a dose-dependent manner by norathyriol, protopine, osthole and magnolol, but not by apigenin. However, the antiplatelet agents (up to 200 mg kg $^{-1}$, i.p.) could not prevent acute thromboembolic death in mice. In endotoxin-induced experimental disseminated intravascular coagulation in rats, norathyriol (50–100 mg kg^{-1}, i.p.) prevented the decrease in platelet counts and fibrinogen, and the prolongation of plasma prothrombin time. Norathyriol (100 mg kg^{-1}, i.p.) also suppressed ex-vivo platelet aggregation induced by collagen and ADP in rat plasma.

Chinese herbs have been used traditionally as important remedies in oriental medicine. In large scale screening tests, we have recently isolated some antiplatelet agents from these herbs. Apigenin from *Apium graveolens* (Teng et al 1988b) and magnolol from *Magnolia officinalis* (Teng et al 1988a) are inhibitors of thromboxane formation, while osthole from *Angelica pubescens* (Ko et al 1989a), protopine from *Corydalis* tubers (Ko et al 1989b) and norathyriol from *Tripterospermum lanceolatum* (Teng et al 1989) (Fig. 1) are inhibitors of both phosphoinositide breakdown and thromboxane formation. In this study, we have compared their effects in-vivo, especially the prolongation of bleeding time and prevention of endotoxin-induced disseminated intravascular coagulation (Thomas & Wessler 1964).

Materials and methods

Materials. Magnolol (Fujita et al 1972), osthole (Wu et al 1990), protopine (Matsuda et al 1988) and norathyriol and its acetate (Lin et al 1982) were prepared as described previously. Apigenin, indomethacin, ADP, sodium arachidonate and endotoxin (*E. coli*) were purchased from Sigma Chemical Co., (St. Louis, MO, USA). Thrombin (bovine) was obtained from Park Davis & Co., (Detroit, MI, USA). Simplastin for the plasma prothrombin time test was purchased from General Diagnostics, Division of Warner-Lambert Co., (Durham, NC, USA). Sodium pentobarbitone was obtained from Tokyo Kasei Kogyo Co., (Tokyo, Japan).

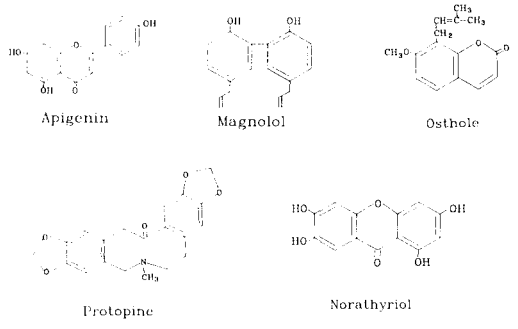

FIG. 1. Chemical structures of the five antiplatelet agents isolated from Chinese herbs.

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Tail bleeding time in conscious mice. Thirty min after the intraperitoneal (i.p.) administration of drugs, mice were placed in a tube holder with the tail allowed to protrude. The tail was transected at 1·5 mm from the tip and 1·5 cm of the distal portion was vertically immersed into saline at 37°C and the bleeding time was measured as described by Hornstra et al (1981).

Acute pulmonary thromboembolic death in mice. Acute pulmonary thromboembolism was induced in mice by a rapid intravenous injection of ADP (400 mg kg^{-1}) or sodium arachidonate (90 mg kg^{-1}) in 3 s according to the method of Nordoy & Chandler (1964).

Endotoxin-induced haematological changes in rats. Thirty min after the administration of drugs (i.p.), endotoxin (20 mg kg^{-1}) was intravenously injected. The rats were anaesthesized by an i.p. injection of sodium pentobarbitone (30 mg kg^{-1}), and following a midline laparotomy, blood was withdrawn from the abdominal aorta into a siliconized tube containing 3·8% sodium citrate. The anticoagulated blood was equally divided into two tubes. One tube was centrifuged, at 90 *g*, room temperature (22°C), 10 min, to obtain platelet-rich plasma (PRP). The other tube was centrifuged at 1500 *g*, 4°C, 30 min, to obtain platelet-poor plasma (PPP). Platelet numbers in PRP were counted with a Coulter counter (Model ZM). For the analysis of fibrinogen in PPP, the method of Ware et al (1947) was used. Plasma prothrombin time of PPP was determined with a coagulometer (General Diagnostics) using Simplastin reagent according to the procedure described by the manufacturer.

Platelet aggregation. PRP was obtained 30 min after the administration of drugs. Aggregation was measured at 37°C by the turbidimetric method of O'Brien (1962) using a Chrono-Log Lumi-aggregometer. PRP samples were stirred for 1 min, 1200 rev min^{-1} before collagen or ADP was added. Percentage aggregation was calculated from absorbance assuming the absorbance of PRP before addition of collagen or ADP corresponded to 0% aggregation and the absorbance of PPP to 100% aggregation.

Results

Effects on tail bleeding time of mice. Of the five antiplatelet agents, norathyriol showed the most marked prolongation of the tail bleeding time in mice (Table 1).

Table 1. Effect of indomethacin, apigenin, osthole, protopine, magnolol, norathyriol, and norathyriol acetate on mice tail bleeding time.

Dose (mg kg^{-1})	Bleeding time (s)								
	Saline	Dimethyl-sulphoxide	Indomethacin	Apigenin	Osthole	Protopine	Magnolol	Norathyriol	Norathyriol acetate
	65·3 ± 5·4 (11)	67·5 ± 5·4 (16)							
100				84·4 ± 16·5 (7)					
30				115·0 ± 17·1* (7)	523·8 ± 37·0*** (6)		493·1 ± 62·4*** (7)	> 600*** (8)	377·7 ± 62·3*** (15)
15					336·9 ± 62·6*** (13)				
10			> 600*** (8)	109·3 ± 19·9** (10)	121·2 ± 56·6 (9)	504·3 ± 61·3*** (11)	243·6 ± 58·7** (14)	518·2 ± 53·9*** (9)	212·8 ± 41·3* (19)
3			507·3 ± 58·8*** (9)	102·7 ± 10·8** (12)		310·8 ± 53·0*** (19)		308·9 ± 60·1*** (17)	122·9 ± 32·9 (16)
1			440·2 ± 45·9*** (22)	83·3 ± 11·1 (10)		194·2 ± 54·2* (16)		240·1 ± 66·8* (9)	
0·3			247·8 ± 59·6* (16)					197·1 ± 67·1* (10)	
0·1			110·9 ± 39·9 (13)						

Drugs were administered i.p. 30 min before test. Values are expressed as means ± s.e.m. The numbers of experiments are in parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the respective control (dimethylsulphoxide-treated) values.

Effects on acute pulmonary thromboembolic death in mice. The mortality ratios after ADP- and arachidonate-induced pulmonary thromboembolism were not reduced by apigenin (50 mg kg^{-1}), osthole (200 mg kg^{-1}) or norathyriol (200 mg kg^{-1}). Indomethacin (50 mg kg^{-1}) had no effect on mortality by sodium arachidonate but significantly reduced mortality at a dose of 200 mg kg^{-1} (Table 2).

Table 2. Effect of apigenin, osthole, norathyriol, norathyriol acetate and indomethacin on acute thromboembolic death in mice.

	Dose (mg kg^{-1})	Mortality induced by ADP	Mortality induced by Sodium arachidonate
Control		14/15	10/10
Indomethacin	50		8/8
	200		6/11*
Apigenin	50	6/6	6/6
Osthole	200	6/6	6/6
Norathyriol	200	6/6	6/6
Norathyriol acetate	200	11/13	6/6

Indomethacin, apigenin, osthole, norathyriol or norathyriol acetate was administered i.p. 30 min before test. Data are presented as (number of mice dead)/(number of mice used), and were analysed by the χ^2-test. * $P < 0.05$ compared with the corresponding control.

Effects on the endotoxin-induced haematological changes in rats. Endotoxin (20 mg kg^{-1}) caused a decrease of platelet counts (thrombocytopenia) and fibrinogen (defibrination) and also a prolongation of prothrombin time (anticoagulation). These endotoxin-induced haematological changes were partially prevented by norathyriol (100 mg kg^{-1}). Norathyriol (50 mg kg^{-1}) inhibited the change in platelet counts, but had no effect on fibrinogen level or prothrombin time (Table 3).

Ex-vivo effect of norathyriol on platelet aggregation. Norathyriol (100 mg kg^{-1}, i.p.) suppressed the aggregation of platelet-rich plasma induced by collagen and ADP (Fig. 2). This inhibition could be observed at a wide range of concentrations of collagen (5–20 μg mL^{-1}) and ADP (1–8 μM).

Discussion

Apart from apigenin the antiplatelet agents isolated from Chinese herbs prolonged the tail bleeding time in mice in a manner similar to other antiplatelet drugs, such as aspirin and indomethacin. However, three of these agents, apigenin, osthole and norathyriol, were completely ineffective at reducing experimental thromboses. Tomikawa et al (1978) reported that aspirin was also ineffective against ADP-induced thromboembolism,

Table 3. Effects of norathyriol on endotoxin-induced haematological changes in rats injected with 20 mg kg^{-1} endotoxin.

Treatment	Dose (mg kg^{-1})	No. of rats	Blood platelets (× 10^4 mm^{-3})	Fibrinogen (mg dL^{-1})	Prothrombin time(s)
Without endotoxin		9	96·7 ± 3·6	170·4 ± 7·5	13·8 ± 0·2
With endotoxin					
Control		12	38·0 ± 4·6	88·5 ± 8·4	18·7 ± 0·9
Norathyriol	50	9	58·1 ± 7·0*	99·7 ± 12·5	16·4 ± 0·4
Norathyriol	100	8	71·1 ± 4·8***	130·0 ± 8·7**	16·0 ± 0·4*

Dimethyl sulphoxide or norathyriol was administered i.p. 30 min before endotoxin. Values are presented as means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the respective control values.

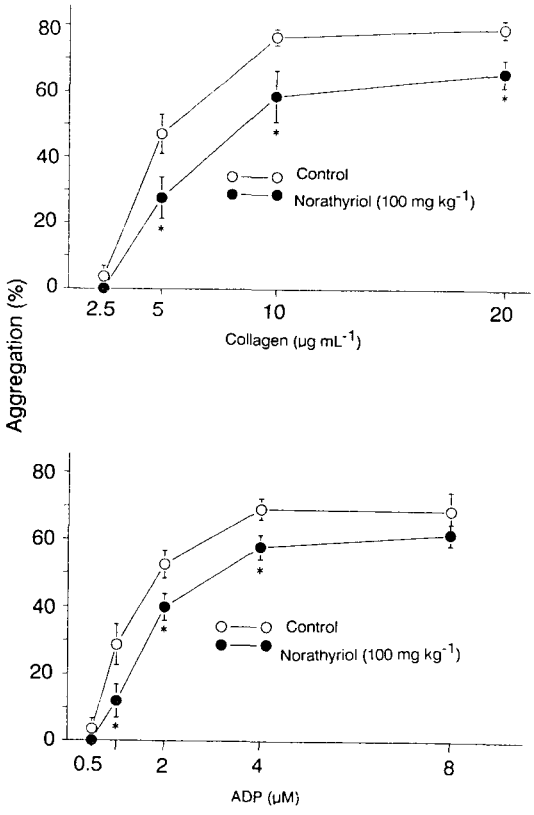

FIG. 2. Ex-vivo effect of norathyriol on the platelet aggregation induced by collagen and ADP. Norathyriol (100 mg kg^{-1}) was administered i.p. to rats 30 min before blood sampling with sodium citrate as anticoagulant. Platelet aggregation was measured in platelet-rich plasma at 37°C using various concentrations of collagen or ADP. Percentages of aggregation are expressed as means ± s.e. Statistically significant differences from the corresponding control values are noted as * $P < 0.05$.

and in the present study in arachidonate-induced acute thromboembolism, indomethacin was effective only at a dose of 200 mg kg^{-1}. Norathyriol was effective in preventing the endotoxin-induced haematological changes, such as thrombocytopenia, defibrination and anticoagulation, and because norathyriol is an antiplatelet agent without anticoagulant action, it is proposed that platelets may play a role in the endotoxin-induced coagulation. Norathyriol was also effective in preventing the ex-vivo aggregation of platelet-rich plasma induced by collagen and ADP. However, the dose required for this prevention was much higher than that which caused prolongation of bleeding time. In addition to its antiplatelet action, norathyriol is also a strong vasorelaxant in rat thoracic aorta (Ko et al 1991). It is a calcium-channel blocker of the vascular smooth muscle. Thus, the vasorelaxing action of norathyriol may synergize with its antiplatelet action and lead to a strong antihaemostatic effect.

Recent experiments have shown that agents isolated from Chinese herbs also caused vasorelaxing actions in rat thoracic aorta. Magnolol relaxes the smooth muscle by releasing endothelium-derived relaxing factor (EDRF) and suppressing calcium influx (Teng et al 1990). Apigenin is an inhibitor of voltage-dependent calcium channels while osthole increases the cGMP levels of the vascular smooth muscle cells. In conclusion, the antihaemostatic effects of these Chinese herbal principles can be explained by their in-vitro antiplatelet and vasorelaxing actions. Further experiments are needed to evaluate their potential as antithrombotic agents.

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