Tissue Factor Inhibitory Triterpenoids

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1. Introduction

Tissue factor(TF) is a cell surface receptor for blood coagulation factor VII(FVII) and is the principal initiator of the vertebrate coagulation cascade.[1] Vascular damage exposes blood to cells expressing TF, which participates to form a TF/FII (a)/phospholipid/Ca^{2+} complex. The complex mediates the activation of both the intrinsic and the extrinsic pathways.[2]

TF is the member of the class 2 cytokine receptor superfamily.[3] Constitutively high levels of TF are found in certain organs such as brain, lung and kidney. Recent studies allowed the more precise localization of TF to outer plasma membrane of various tissue cells such as vascular adventitia, organ capsules, epithelium of skin, and mucosa. Bowman`s capsule of glomeruli in the kidney, astrocytes in the brain, and stromal cell of human endometrium. Blood monocytes, fibroblasts and macrophages have been found to express TF constitutively, when quiescent, only trival amounts of TF are expressed. TF antigen can be induced in various cell types often challenging a wide variety of stimulators such as endotoxins, tumor necrosis factor-α, phobol esters, growth factors, lipopolysaccharide, lymphokines, immune complexes and terpentine oil.

It was observed that TF antigen and activity could be isolated in the whole blood and plasma from certain diseases and healthy volunteers. Given the evidence for an involvement of TF/VIIa in the pathophysiology of thrombosis and sepsis, potent and selective inhibitors have been sought for intervention in these diseases. Inhibitory monoclonal antibodies raised against TF have been shown to have antithrombotic activity in animal models of thrombosis. Significantly, an anti-TF antibody had an antithrombotic effect but did not cause an increased bleeding tendency. Anticoagulant potency appears to be linked to the binding epitope. Atibodies that bind to the putative substrate contact region on TF are more effective than antibodies that block the FVIIa binding site. Because monoclonal antibodies have long serum half-lives, an anti-TF antibody had potential for the treatment of postsurgical thrombosis, where a long-acting agent is needed.

Tissue factor pathway inhibitor (TFPI) is known to be a multivalent, Kunitz-type plasma proteinase inhibitor that regulates TF-induced coagulation. TFPI directly inhibits Xa and, in a factor Xa-dependent fashion, produces feedback inhibition of the TF/VIIa catalytic complex.

This critical position of the TF/ VII(a) complex in the blood coagulation cascade makes it an attractive target for anti-coagulant drug discovery. Thus researches of TF inhibitors derived from natural products are supposed to have a profound significance. We have screened various medicinal plants belonging to Aquifoliaceae and Rosaceae for examining their inhibitory effects on TF by utilizing some screening methods such as intrinsic and extrinsic anticoagulant and antiplatelet activities, *in vivo* and *in vitro* (Table 1).

In the first study step to screen the antithrombotic activity, we have measured the bleeding time (BT), the whole blood recalcification clotting time (WRCT), and the plasma recalcification clotting time (PRCT), successively, after orally administering an extract of herbal medicines to rats. An intrinsic pathway anticoagulant drug should prolong all the three kinds of the barometers, an extrinsic pathway anticoagulant drug should prolong only BT, and antiplatelet drug should prolong both BT and WRCT.

In the second study step to isolate active principles, we have measured *in vitro* the inhibitory activities on PRCT, prothrobin time (PT) and platelet aggregation (PA) for the intrinsic pathway anticoagulant, the extrinsic pathway anticoagulant and the antiplatelet activities, respectively. We have isolated several triterpenoids with TF inhibitory activity, and here we introduce their chemical structures and activities.

Table 1. Screening methods for antithrombotic activity

Activity	*In vivo*			*In vitro*		
	BT	WRCT	PRCT	PRCT	PT	PA
• Anticoagulant						
- Intrisic	O	O	O	O	O	X
- Extrinsic	O	X	X	X	O	X
• Antiplatelet	O	O	X	X	X	O

O or X , effective (prolong or inhibit) or non-effective ; BT, bleeding time; WRCT, whole blood recalcification clotting time; PRCT, plasma recalcification clotting time; PT, prothrombin time; PA, platelet aggregation.

2. *Ilex pubescens* roots (毛冬青)

The root or leaf of *Ilex pubescens* Hook. et Arn (Aquifoliceae), 'Maodongqing(毛冬青)', is widely used in China for the treatment of cardiovascular diseases, cerebral thrombosis, thromboantiitis oblitersans, *etc.* An *in vivo* study in rats, the methanol extract of the roots prolonged BT and inhibited PA induced by thrombin, but did not affect PRCT. Its saponin named ilexoside was proved to be responsible for the antithrombolic activities.[4] Ilexoside A, D, and J, of which genin is pubescenolic acid (=20 epipomolic acid), and pubescenic acid (=24-carboxyl pomolic acid) showed the strong inhibitory activities on PA induced by thrombin (Table 2). Among them, ilexoside D [3-O-β-D-glucopyranosyl (1 2)-β-D-xylopyranoside of pubescenolic acid] showed the most potent antiplatelet activity, which was investigated *in vivo* and *in vitro* models of PA induced by ADP, thrombin or collagen in rats. *In vitro* ilexoside D inhibited more effectively PA induced by ADP and thrombin than by collagen as composed with aspirin. *Ex vivo* ilexoside D also inhibited PA induced by ADP and collagen, but not by thrombin, and the inhibitory action of ilexoside D was more effective than that of aspirin.[5] However, *in vitro* ilexoside D very poorly inhibited PA induced by thrombin. The antithrombotic activity of ilexoside D was also investigated in *in vivo* and *in vitro* models of blood coagulation in rats. On oral administration, ilexoside D prolonged BT and WRCT, but not PRCT. *In vitro*, ilexoside D did not affect the recalcification clotting times of whole blood (WRCT), platelet-rich plasma, and platelet-poor plasma (PRCT), while in the presence of TF the compound prolonged PT of whole blood, PRP and PPP in a dose-dependent manner. These results indicate that ilexoside D has the anti-TF activity as well as the antithrombotic activity.[6,7]

Ilexosides and their aglycones, pubescenolic acid, pubescenic acid, and ilexolic acid (18,19-dehydro-pomolic acid) were reinvestigated in the aspect of TF inhibitory activity. Table 2 shows that ilexoside D (IC_{50}, 52.1 μM) exhibited weaker TF inhibitory activity than its aglycone pubescenolic acid(IC_{50}, 0.80 μM), ilexoside A(3-O-β-D-xylopyranosyl-28-O-β-D-glucopyranoside of pubescenolic acid) (IC_{50}, 23.0 μM) and its prosapogenin A (IC_{50}, 3.79 μM). There results indicated that ilexosides possesses the anti TF activity as well as antiplatelet activity.

3. *Sanguisorba officinalis* roots (地楡)

The root of *sanguisorba officinalis (*Rosaceae*)* was proved by us to contain ziyu-glycoside I as the principle of the antithrombotic activity, and to contain ziyu- glycoside Ⅱ and its aglycone, pomolic acid as the principles of the anti-TF activity (Table2). Ziyu-glycoside Ⅰ and Ⅱ are bis-and mono-desmoside of pomolic acid, respectively. The antiplatelet activity was only found in ziyu-glycoside Ⅰ, whereas the anti-TF activity was in ziyu-glycoside Ⅱ (IC_{50}, 0.26 μM) and pomolic acid (IC_{50}, 0.45 μM). Ziyu-glycoside Ⅱ, one of the most strong TF inhibitor found by us until now, was effective *in vitro,* but not *in vivo*. Pomolic acid derivatives such as tormentic acid (2α-form of pomolic acid) and rotundic acid (23-hydroxy form of pomolic acid) did not inhibit the TF procoagulant activity *in vitro.*

Thus, we have prepared the derivative of ziyu-glycoside Ⅱ named ZYM201, which strongly inhibited the rat brain TF activity *in vivo.*

4. *Chaenomeles sinensis* (木瓜)

From the fruit of C*haenomeles sinensis* (Rosaceae) a new saponin, named chaenomeloside A and its aglycone, named chaenomelogeninA (=2α-hydroxy-24-carboxyl oleanolic acid) were isolated, and their TF inhibitory activity was measured as 36.0 and 28.0 μM *in vitro,* respectively.[8)]

Table 2 : Antithrombotic acid and anticoagulant activities of ilexosides and their aglycones isolated from *Ilex pubescense* (A,B and C), *Sanguisorba officinalis* (D) and *Chaenomeles sinensis* (E)

Aglycone / its glycoside	Antithrombotic IC_{50} (mg/10^9 platelet)	Anti-tissue factor IC_{50} (μM/ TF unit)
A. Pubescenolic acid	NA*	0.80
Ilexoside A	0.22	20.30
Prosapogenin A	NA	3.79
Ilexoside D	0.17	52.10
Ilexoside J	0.68	NA
Ilexoside K	NA	NA
Ilexoside O	NA	NA
A. Pubescenic acid	0.41	23.48
Ilexoside E	NA	NA
C. Ilexolic acid	NA	NA
Ilexoside H	NA	NA
Prosapogenin H	NA	NA
D. Pomolic acid	NA	
Ziyu-glycoside I	0.26	0.45
Ziyu-glycoside II	NA	NA
		0.26
E. Chaenomelogenin A	NA	
Chaenomeloside A	NA	28.0
		36.0

*NA:non-Active

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