REVIEW ARTICLE

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Platelet Activation, and Antiplatelet Targets and Agents

Current and Novel Strategies

Yao-Zu Xiang, Ye Xia, Xiu-Mei Gao, Hong-Cai Shang, Li-Yuan Kang and *Bo-Li Zhang*

Research Center of Tianjin University of Traditional Chinese Medicine, Tianjin, China

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Abstract

Platelets play a key role in thrombosis and haemostasis, which can be either beneficial or deleterious depending on the circumstances. Multiple factors, such as genetic polymorphisms, pathological state and lifestyle, are thought to be associated with platelet hyperreactivity. Platelet activation occurs through the complex process of transmembrane signalling, with a cascade of biochemical interactions leading to platelet activation. Transmembrane signalling involves many different molecules with different enzymatic activity and/or function. Based on the signalling pathways involved in platelet activation, there are four possible targets of antiplatelet drugs: (i) inhibition of agonist generation; (ii) receptor inhibition; (iii) G-protein inhibition; and (iv) inhibition of enzymatic cascades. However, both established and novel antiplatelet drugs have their own advantages and disadvantages. Because of the problems associated with the use of current antiplatelet drugs, such as resistance, optimal dosage and safety, future strategies

for the development of new antiplatelet drugs and new treatment regimens may include consideration of the following: (i) a shift from single targets within the signalling cascade to multiple targets; (ii) a shift from therapy with a single drug to combination therapy; and (iii) investigating drugs in current clinical use for novel antiplatelet properties.

Coronary artery disease (CAD) and stroke continue to be the most common causes of mortality and morbidity in the industrialized world. Thrombotic diseases affect the cardiovascular, cerebrovascular and peripheral arterial systems. Platelets play a key role in both thrombosis and haemostasis but, under normal physiological conditions, their major physiological function is haemostasis (i.e. the maintenance of vessel integrity and the cessation of bleeding following injury). However, under pathophysiological conditions, platelet activation leads to a range of responses that play a critical role in arterial thrombosis, including platelet aggregation, the secretion of dense and α granules, and procoagulant activity.[1-3] To some extent, the balance between thrombosis and haemostasis is similar to the Chinese medical theory of Yin and Yang (see figure 1). As the number of factors recognized as being associated with platelet hyperactivity increases and the related mechanisms are clarified, more antiplatelet agents can be developed. However, the treatment of thrombotic disease requires a delicate balance between the prevention of new thrombotic events and the management of bleeding complications. To this end, various antiplatelets have been used with varying degrees of success.

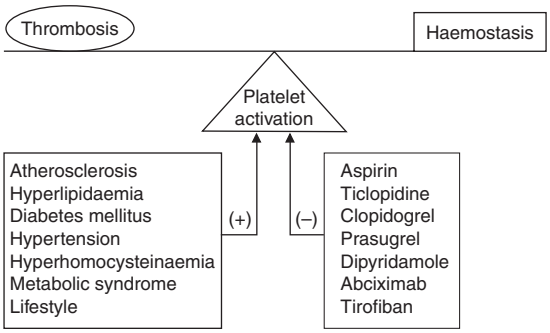

Fig. 1. The Yin and the Yang of thrombosis and haemostasis. *In vivo*, there is a balance between thrombosis and haemostasis. Many factors increase platelet activation (+) and are thus associated with platelet hyperreactivity, including atherosclerosis, hyperlipidaemia, hypertension and lifestyle. Currently available antiplatelet agents, including aspirin and clopidogrel, have inhibitory effects (–) on platelet activation.

On the basis of results of gene-knockout studies and with the availability of multiple receptor antagonists, details of the process of platelet activation have become clearer. Platelet activation is a complicated process, involving multiple signalling pathways and molecules with various enzymatic activities and/or function.[3] Thus, it may be difficult to study platelet signalling from the perspective of a single ligand–receptor interaction. However, this aspect is very important for an understanding of the mechanisms underlying platelet activation with different agonist-mediated signalling pathways, which is the basis for research into potential new targets for antithrombotic drugs. The present review summarizes factors associated with platelet hyperreactivity, recent advances in the understanding of the main agonist-mediated signalling pathways involved in platelet activation, the problems associated with the use of current antiplatelet drugs and future considerations.

1. Factors Associated with Platelet Hyperreactivity

Epidemiological studies have linked platelet hyperreactivity with an increased risk of vascular events. Even more convincing evidence of such a link comes from appropriately designed clinical trials, which have shown that the use of antiplatelet agents decreases the risk of vascular events (e.g. myocardial infarction [MI] and stroke).[4] These findings are compatible with the known thrombotic actions of platelets. However, it is difficult to reliably identify a 'high-risk' patient because of the absence of a reliable, universally accepted marker of platelet activity. Recently, other factors, such as genetic polymorphisms, pathological state and lifestyle, have been identified as contributing to platelet

hyperreactivity and some of the mechanisms involved have been determined.[4]

1.1 Genetic Polymorphisms

The genetic contribution to thrombotic diseases is well established.[5,6] Genetic polymorphisms may affect the balance between coagulation and fibrinolysis, thereby impacting on vulnerability to acute MI among patients with underlying coronary atherosclerosis. Although there are well known examples of inherited gene variations that affect platelet function and lead to a clinical haemorrhagic phenotype (e.g. Glanzmann thrombasthenia and storage pool diseases), only recently have investigators started to consider inherited variations that enhance platelet reactivity. Since the first report of genetic variations in platelets associated with acute MI in 1996,[7] many epidemiological studies have been undertaken, but the results have often been inconsistent.[8] It has been determined that 10 of 11 polymorphisms in eight platelet genes are not associated with platelet hyperreactivity, including functional polymorphisms in genes encoding the α_2-adrenoceptor, integrins $\beta 3$ and $\alpha 2$, and glycoprotein (GP) Iba and GP VI. However, platelet hyperreactivity has been shown to be associated with the C825T polymorphism in the gene encoding the G-protein $\beta 3$-subunit.[9] Although polymorphisms of most platelet genes are not related to platelet hyperreactivity, there are genetic polymorphisms that are associated with platelet hyperreactivity and are considered responsible for resistance to antiplatelet agents (e.g. aspirin[10-13] and clopidogrel[14-17]).

1.2 Pathological State

1.2.1 Atherosclerosis and Vascular Events

Normal platelet responses can be altered by active cardiovascular disease states, both chronic (e.g. stable angina pectoris) and acute (e.g. acute MI). For example, the generation of thromboxane A_2 (TXA_2) was found to be increased in patients with an acute MI, whereas patients with acute coronary syndromes (ACS) exhibited significantly enhanced GP VI expression.[18] In addition, platelet reactivity has been shown to be increased in patients with peripheral arterial disease.[19,20]

1.2.2 Hyperlipidaemia

Platelet hyperreactivity can be assessed with multiple stimuli in multiple assays and is more likely to be present in individuals who have elevated levels of fibrinogen, triglycerides and low-density lipoproteins (LDLs).[21-23]

The interaction between platelets and lipoproteins has been investigated in some depth. Recent observations suggest that LDL enhances platelet responsiveness.[23] Several LDL-induced signalling pathways involved in platelet activation have been revealed *in vitro*, including signalling via p38 mitogen-activated protein kinase and p125 focal adhesion kinase.[24,25] High-density lipoprotein (HDL), which consists of two subtypes, namely HDL2 and HDL3, has opposite effects to LDL on platelet activation.[23]

Interestingly, it was found that spontaneous platelet aggregation in whole blood was increased in female but not male patients with primary dyslipidaemia, indicating that higher shear-induced aggregation may exist in patients with hyperlipidaemia, leading to thrombus formation.[26] Moreover, no influence of simvastatin was found on platelet aggregation and secretion in patients with hypercholesterolaemia.[27]

1.2.3 Diabetes Mellitus

Diabetes mellitus is a well recognized risk factor for atherosclerotic cardiovascular disease and is, in fact, associated with enhanced platelet activation. Diabetic patients have hyperreactive platelets that exhibit exaggerated adhesion and aggregation, as well as increased thrombin generation (i.e. the entire coagulation cascade is dysfunctional in diabetes).[28] A variety of mechanisms may be responsible for enhanced platelet aggregation in diabetes.[29-31] First, hyperglycaemia may be related to the nonenzymatic glycation of platelet glycoproteins, resulting in changes in structure and conformation, as well as changes in membrane lipid dynamics. Second, hyperglycaemia-induced oxidative stress is responsible for the enhanced peroxidation of arachidonic acid, leading to the formation of biologically active isoprostanes. Moreover, increased oxidative stress contributes to activation of transcription factors and the expression of redox-sensitive genes, leading to initial platelet activation and adhesion, with subsequent formation of platelet aggre-

gates. Third, high glucose levels enhance platelet reactivity to agonist stimulation as a result of elevated osmolality. This effect is mediated by the production of superoxide anion, which increases platelet expression of P-selectin, and protein kinase C signalling, which intensifies thrombin receptor-activating peptide (TRAP)-induced fibrinogen binding (i.e. aggregability).[29] Finally, studies have shown that food intake enhances thromboxane receptor-mediated platelet activation in patients with type 2 diabetes, but not in healthy subjects.[31]

1.2.4 Hypertension

In general, results of clinical studies and animal experiments indicate that hypertension is associated with the hyperaggregability of platelets and increased TXA_2 levels in blood, urine and tissues. Platelet activation is involved in the pathogenesis of thrombotic complications of hypertension. There is a stepwise augmentation of platelet activation indices, despite similar platelet counts, with increasing severity of hypertensive disease.[32] A recent study has identified an association between extreme increases in blood pressure (BP) and platelet P-selectin levels and fibrinolytic markers in patients with severe hypertension. Platelet CD62 levels were demonstrated to have a strong and graded association with both systolic and diastolic BP that persisted in the presence of multiple concomitant risk factors. The correlation between BP and CD62P was stronger than that between BP and either plasminogen activator inhibitor-1 activity or tissue plasminogen activator antigen.[33] Platelet activation and platelet CD62 levels increased in a BP-dependent manner and this relationship persisted at extreme levels of BP.[33]

1.2.5 Other Diseases

Epidemiological evidence suggests that hyperhomocysteinaemia may lead to an increase in platelet activation and is an independent risk factor for arterial thrombotic diseases, such as acute MI, stroke, peripheral and ischaemic occlusive disorders and venous thromboembolism.[34-36] Moreover, platelet activation tends to be increased in metabolic syndrome. In overweight and obese patients, the risk factors for metabolic syndrome parallel platelet responsiveness to leptin to some extent.[37]

1.3 Lifestyle

Lifestyle factors, such as exercise, smoking, diet and alcohol consumption, may have a significant effect on cardiovascular disease and platelet function. Physical activity and lifestyle in general have been shown to have diverse effects on platelet reactivity in humans. Many studies have investigated the effects of exercise, weight loss, dietary lipids (especially omega-3 polyunsaturated fatty acids), smoking, alcohol and psychosocial stress on platelet activation. Available evidence supports the theory that adopting strategies to lose weight, stop cigarette smoking, engage in regular moderate exercise and relaxation, as well as the regular light-to-moderate consumption of alcohol and fatty fish, should significantly reduce platelet reactivity.[38] Recent studies have shown that in patients with stable CAD, increased platelet reactivity to collagen/adenosine diphosphate (ADP) stimulation after exercise, as assessed by the PFA-100® System is specifically associated with increased platelet expression of the GP IIb/IIIa receptor.[39] Diet is one factor that influences thrombosis and haemostasis. An increasing number of foods have been reported to have platelet-inhibitory actions, including grape juice, cocoa and chocolate.[40,41] Both epidemiological studies and clinical trials indicate that the very long chain omega-3 fatty acids lower thrombotic tendency and the risk of heart disease. Other polyunsaturated and monounsaturated fats appear to have antithrombotic properties, but further studies are indicated.[42] In a limited number of clinical and laboratory studies, tocopherol (vitamin E) has been shown to decrease platelet aggregation.[43] Nutritional status frequently declines with age and this may exacerbate the already increased risk of thrombosis.[44]

2. Signalling Pathways Involved in Platelet Activation

Platelet activation has long been recognized as critical for the formation of haemostatic plugs and thrombosis. Until relatively recently, platelet activation was considered a straightforward process involving the noncovalent bridging of integrin $\alpha IIb\beta 3$ receptors on the platelet surface by the dimeric adhesive protein fibrinogen. However, with recent technical advances enabling real-time analysis of

Table I. Main platelet agonists and their receptors, G-proteins and relative effectors

Agonists	Receptors	Antagonists	G-protein	Effectors
ADP	$P2Y_1$	A3P5P, MRS2179	G_q	PLCβ (+)
	$P2Y_{12}$	Clopidogrel	G_i	AC (−); PI3K (+)
		Ticlopidine		
		Prasugrel (CS-747)		
		ARL-66096 (AR-C66096), BX 667		
		Regrelor (INS50589)		
Thrombin	PAR1	RWJ-58239, RWJ-56110	G_i; $G_{12/13}$	AC (−); PI3K (+); RhoGEF (+)
	PAR4	YD-3	G_q; $G_{12/13}$	PLCβ (+); RhoGEF (+)
TXA_2	TP receptor	Terutroban sodium (S18886), piperlongumine	G_q; $G_{12/13}$	PLCβ (+); RhoGEF (+)
		BM573, ridogrel, TRA418		
Adrenaline	α2-Adrenoceptor		G_z	AC (−); PI3K (+)
Serotonin	$5\text{-}HT_{2A}$	Sarpogrelate	G_q	PLCβ (+)
PGI_2	IP receptor	RO1138452	G_s	AC (+)
Collagen	GP VI	EXP3179		PLCγ (+)

5-HT = serotonin; **AC** = adenylate cyclase; **ADP** = adenosine diphosphate; **GP VI** = glycoprotein VI; **PAR** = protease-activated receptor; **PGI_2** = prostacyclin; **PLCβ**, **PLCγ** = phospholipase Cβ and γ, respectively; **PI3K** = phosphatidylinositol 3-kinase; **RhoGEF** = Rho guanine-nucleotide exchange factor; **TXA_2** = thromboxane A_2; + indicates positive effect; − indicates negative effect.

platelet activation *in vivo*, it has become apparent that this process is much more complex and dynamic than previously thought. Over the past decade, it has become clear that platelet activation is a multistep process involving distinct receptors and ligands, with the contribution of individual receptor–ligand interactions to the activation process dependent on the prevailing blood flow conditions.[45]

Using gene-knockout studies and multiple receptor antagonists, the details of the process of platelet activation have become much clearer and have been reviewed in detail elsewhere from the viewpoint of platelet physiology,[1,46] signal transduction[47] and G-proteins.[48-50] It is known that platelet activation requires agonist induction. Table I lists the main agonists involved in platelet activation, as well as their receptors, G-proteins and effectors. Because most agonists can act synergistically in platelet aggregation, the signalling pathway of platelet activation is nonlinear and quite complicated (see figure 2). For example, many platelet agonists bind more than one receptor (e.g. von Willebrand factor binds both GP Ib and αIIbβ3, collagen binds to GP VI and α2β1, thrombin interacts with protease-activated receptors and GP Ib, and ADP binds at least two ADP receptors on platelets). In addition, activated platelets themselves rapidly secrete additional agonists (e.g. TXA_2, ADP, serotonin and ATP), which act as positive feedback mediators that amplify the initial signals to ensure the rapid activation and recruitment of platelets into a growing thrombus.

3. Current Antiplatelet Targets and Agents

3.1 Strategies for Antiplatelet Therapy

Based on the signalling pathways involved in platelet activation, there are four possible targets of antiplatelet drugs: (i) inhibition of agonist generation; (ii) receptor inhibition; (iii) G-protein inhibition; and (iv) inhibition of enzymatic cascades. Moreover, some drugs have a clinically demonstrated antiplatelet effect and a clear mechanism of action, for example cyclo-oxygenase (COX) inhibitors (aspirin), phosphodiesterase (PDE) inhibitors (dipyridamole and cilostazol), inhibitors of $P2Y_{12}$ receptors (ticlopidine, clopidogrel and prasugrel) and inhibitors of GP IIb/IIIa receptors (abciximab, eptifibatide and tirofiban).

3.2 Evidence from Clinical Studies on Currently Available Antiplatelet Drugs

Table II summarizes the clinical aspects of currently available antiplatelet drugs and provides a comprehensive list of current English-language systematic reviews and meta-analyses that have

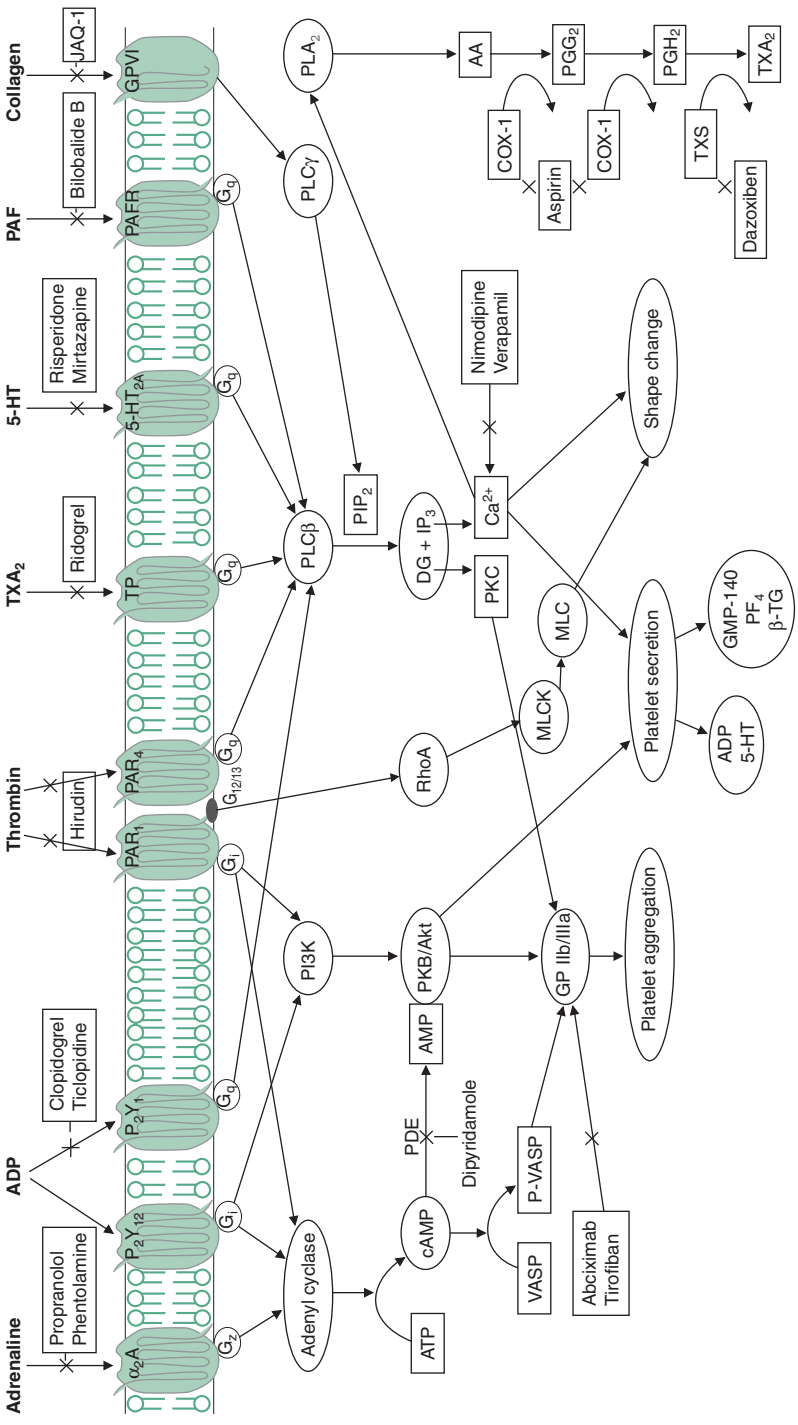

Fig. 2. Signalling pathways involved in platelet activation and on which antiplatelet agents act. **5-HT** = serotonin; **β-TG** = beta-thromboglobulin; **AA** = arachidonic acid; **ADP** = adenosine diphosphate; **AMP** = adenosine monophosphate; **ATP** = adenosine triphosphate; **cAMP** = cyclic AMP; **COX** = cyclo-oxygenase; **DG** = diacylglycerol; **GMP-140** = 140 kDa granule membrane protein (also known as P-selectin); **GP** = glycoprotein; **IP_3** = inositol-1,4,5-trisphosphate; **MLC** = myosin light chain; **MLCK** = myosin light chain kinase; **PAF** = platelet-activating factor; **PDE** = phosphodiesterase; **PF_4** = platelet factor 4; **PGG_2**, **PGH_2** = prostaglandin G_2 and H_2, respectively; **PI3K** = phosphatidylinositol 3-kinase; **PIP_2** = phosphatidylinositol-4,5-bisphosphate; **PKB**, **PKC** = protein kinase B and C, respectively; **PLA_2**, **PLCβ**, **PLCγ** = phospholipase A_2, Cβ and Cγ, respectively; **P-VASP** = phosphorylated VASP; **RhoA** = Ras homolog gene family, member A; **TP** = TXA_2 receptor; **TXA_2** = thromboxane A_2; **TXS** = thromboxane synthase; **VASP** = vasodilator-stimulated phosphoprotein.

Table II. Systematic reviews and meta-analyses of randomized controlled trials (RCTs) of antiplatelet agents

Study (year)	RCTs	Patient population [n (disease/procedure)]	Findings and conclusions
Aspirin			
Efficacy			
Berger et al.[55] (2006)	6	95 456 (CV)	Aspirin reduced the risk of ischaemic stroke in women and MI in men, but significantly increased the risk of bleeding to a similar extent in both women and men
Berger et al.[51] (2008)	6	9853 (CV)	Low-dose (50–325 mg/day) aspirin reduces the incidence of adverse cardiovascular events and all-cause mortality while increasing the risk of severe bleeding
Safety			
Serebruany et al.[61] (2005)	31	192 036 (ACS)	Low-dose (<100 mg) aspirin provides comparable therapeutic effects with the lowest risk, whereas moderate doses (100–200 mg) are associated with a higher rate of haemorrhagic events
Biondi-Zoccai et al.[62] (2006)	6[a]	50 279 (CAD)	Aspirin withdrawal was associated with 3-fold higher risk of major adverse cardiac events
Burger et al.[63] (2005)	41[a]	49 590 (ACS)	Aspirin could be discontinued peri-operatively only if peri-operative bleeding risks are similar or more severe than the observed cardiovascular risks after withdrawal
Aspirin resistant			
Krasopoulos et al.[64] (2008)	20[a]	2930 (CV)	28% of patients were classified as aspirin resistant, did not benefit from other antiplatelet treatment and were at a greater risk of cardiovascular morbidity
Hovens et al.[65] (2007)	42[a]	NR	Biochemically defined aspirin resistance is approximately 25%. Both aspirin dosage and the method of defining aspirin resistance strongly influence estimated prevalence
Snoep et al.[66] (2007)	16[a]	3304 (CV)	Patients identified biochemically as having laboratory aspirin resistance are more likely to also have 'clinical resistance' (OR 3.8; 95% CI 2.3, 6.1)
Clopidogrel			
Lotrionte et al.[56] (2007)	10[a]	1567 (PCI)	A high clopidogrel loading dose (>300 mg) significantly reduces early ischaemic events in patients scheduled for PCI
Snoep et al.[67] (2007)	25[a]	3688 (PCI)	Mean prevalence of clopidogrel nonresponsiveness was 21% and the pooled OR of cardiovascular outcome was 8.0
Purkayastha et al.[68] (2006)	11[a]	4002 (CABG)	Clopidogrel may indirectly compromise hospital resources by increasing blood loss, transfusion, ventilation requirements, length of inpatient stay and re-exploration rate
Cilostazol			
Thompson et al.[57] (2002)	8	2702 (IC)	Cilostazol therapy increased maximal and pain-free walking distances by 50% and 67%, respectively, without major adverse effects
Robless et al.[58] (2007)	8	NR (IC)	Cilostazol has been shown to be of benefit in improving walking distance in people with IC
Dipyridamole			
Leonardi-Bee et al.[59] (2005)	5	11 459 (stroke)	Recurrent stroke was reduced by dipyridamole vs control (OR 0.82) and by combined aspirin and dipyridamole vs aspirin (0.78), dipyridamole (0.74) or control (0.61)
De Schryver et al.[69] (2003)	26[a]	19 842 (stroke)	There was no evidence that dipyridamole alone was more efficacious than aspirin, although it may reduce the risk of further vascular events
De Schryver et al.[53] (2008)	29	23 019 (VD)	There was no evidence that dipyridamole alone is more efficacious than aspirin

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Table II. Contd

Study (year)	RCTs	Patient population [n (disease/procedure)]	Findings and conclusions
Triflusal			
Costa et al.[54] (2006)	7[a]	5622 (stroke)	No significant differences were found between triflusal and aspirin for secondary prevention of serious vascular events, but triflusal was associated with a lower risk of haemorrhagic complications
Abciximab			
De Luca et al.[70] (2006)	7	3918 (STEMI)	The mortality benefits of adjunctive abciximab therapy to mechanical revascularization for STEMI are related to the patient's risk profile
Montalescot et al.[60] (2007)	3	1101 (stenting)	Abciximab has a strong and persistent impact on hard clinical endpoints in patients undergoing primary stenting for STEMI
de Queiroz Fernandes Araújo et al.[71] (2004)	6	3755 (PCI)	Abciximab, as adjunctive therapy to PCI, reduces mortality, TVR and MACE following AMI, with an increased risk of major bleeding (OR 1.39; 95% CI 1.03, 1.87)
Kandzari et al.[72] (2004)	4	3266 (PCI)	Treatment with abciximab significantly reduces early adverse ischaemic events, a clinical benefit that is maintained at 6-month follow-up
GP IIb/IIIa antagonists			
Hernández et al.[73] (2007)	6	31 402 (NSTE-ACS)	The relative reduction of death or MI with GP IIb/IIIa inhibitors was greater in older patients, but with a higher risk of major bleeding
Montalescot et al.[74] (2004)	6	931 (STEMI)	Early administration of GP IIb/IIIa inhibitors appeared to improve coronary patency with favourable trends for clinical outcomes, supporting a strategy of facilitated PCI

a Including non-RCTs.

ACS = acute coronary syndromes; **AMI** = acute myocardial infarction; **CABG** = coronary artery bypass grafting; **CAD** = coronary artery disease; **CI** = confidence interval; **CV** = cardiovascular disease; **GP** = glycoprotein; **IC** = intermittent claudication; **MACE** = major cardiac events; **MI** = myocardial infarction; **NR** = not reported; **NSTE** = non-ST-segment elevation; **OR** = odds ratio; **PCI** = percutaneous coronary interventions; **STEMI** = ST-segment elevation myocardial infarction; **TVR** = target vessel revascularization; **VD** = vascular disease.

been published in peer-reviewed journals and retrieved from MEDLINE, PsycINFO, Acular and AMED, as well as a compilation of published bibliographies. Based on these data, low-dose aspirin (50–325 mg/day) is still the most effective treatment to reduce the risk of nonfatal MI and all-cause mortality among patients with ischaemic heart disease. In addition, aspirin is the most effective at reducing the risk of stroke among patients with cerebrovascular disease, although it does increase the risk of severe bleeding.[51] However, one systematic review indicates that currently available clinical data do not support the routine long-term use of aspirin at dosages greater than 75–81 mg/day in the prevention of cardiovascular disease.[52] Higher dosages of aspirin do not reduce the risk of vascular events but, rather, are associated with an increased risks of gastrointestinal bleeding. There is no evidence that dipyridamole alone or triflusal alone are more efficacious than aspirin.[53,54] The protective effects of aspirin are sex specific,[55] but it is unclear whether other antiplatelet drugs have similar properties. If an analogy can be made of aspirin as a broad-spectrum antibacterial, then the other antiplatelet drugs currently available can be viewed as narrow-spectrum antibacterials. Because of a lack of sufficient clinical evidence, current clinical data support the use of aspirin in primary and secondary thrombotic diseases, clopidogrel in ACS and percutaneous coronary interventions (PCI), cilostazol in intermittent claudication, triflusal and dipyridamole in stroke, and GP IIb/IIIa inhibitors in ACS and stenting.[51,54,56-60] However, this does not mean that an antiplatelet agent that is recommended for the treatment of a certain disease cannot be used for the treatment of other thrombotic conditions.

Table III lists studies comparing the clinical efficacy and safety of available antiplatelet agents. These studies indicate that prasugrel is superior to clopidogrel, which itself is superior to aspirin, in preventing vascular death, MI and stroke,[75-77] whereas abciximab seems to be more effective than tirofiban in the prevention of ischaemic events with percutaneous coronary revascularization.[78]

Table IV lists studies on combination therapy using currently available antiplatelet drugs. The

Table III. Comparison of the clinical efficacy and safety of currently available antiplatelet agents

Agents	Study	Patient (n)	Treatment (mg/day)	Follow-up	Primary endpoint	RRR (incidence of combined endpoint) [%]
Triflusal vs aspirin	TACIP[79]	2 113	600 vs 325	Mean 30.1 mo	Nonfatal stroke, nonfatal AMI or vascular death Major haemorrhage	(13.1 vs 12.4)* 33.7 (16.7 vs 25.2)
	Cruz-Fernández et al.[80]	2 275	600 vs 300	35 d	Death, nonfatal myocardial reinfarction or a nonfatal cerebrovascular event	(9.06 vs 10.15)*
	TAPIRSS[81]	429	600 vs 325	Mean 586 d	Vascular death, cerebral, infarction, nonfatal MI, major haemorrhage	(12.7 vs 13.9)*
Clopidogrel vs aspirin	CAPRIE[75]	19 185	75 vs 325	1–3 y	Vascular death, MI or ischaemic stroke	8.7 (5.32 vs 5.83)
	CAPRIE[76]	19 185	75 vs 325	1–3 y	AMI	19.2 (4.2 vs 5.04)
Ticlopidine vs aspirin	STAMI[82]	1 470	500 vs 160	6 mo	AMI, stroke, angina, vascular death	(8.0 vs 8.0)*
Ticlopidine vs clopidogrel	Taniuchi et al.[83]	1 016	500 vs 300	30 d	Subacute stent thrombosis	4.95 (1.92 vs 2.02)*
Prasugrel vs clopidogrel	TRITON-TIMI 38[77]	13 608	10 vs 75	6–15 mo	CV death, nonfatal MI or nonfatal stroke Major bleeding	18.2 (9.9 vs 12.1) (2.4 vs 1.8)
	PRINCIPLE-TIMI 44[84]	201	LD: 60 vs 600	6 h	IPA	(74.8 ± 13.0 SD vs 31.8 ± 21.1 SD)
			MD: 10 vs 150	28 d	IPA	(61.3 ± 17.8 SD vs 46.1 ± 21.3 SD)
	JUMBO-TIMI 26[85]	904	LD: 60 vs 300, MD: 10 vs 75	30 d	Bleeding events	(1.7 vs 1.2)*
AZD6140 vs clopidogrel	DISPERSE-2[86]	990	180 vs 75 360 vs 75	12 wk	Major or minor bleeding	(9.8 vs 8.1)* (8.0 vs 8.1)*
Abciximab vs tirofiban	TARGET[78]	4 809	LD: 0.25 mg/kg vs 10 μg/kg, MD: 0.125 μg/kg vs 0.15 μg/kg	30 d	Death, nonfatal MI, revascularization	21.1 (6.0 vs 7.6)

AMI = acute myocardial infarction; **CV** = cardiovascular disease; **IPA** = inhibition of platelet aggregation; **LD** = loading dose; **MD** = maintenance dose; **MI** = myocardial infarction; **RRR** = relative risk reduction; * $p > 0.05$.

Table IV. Outcomes of combination therapy using currently available antiplatelet agents

Agents	Study	Patient population [n (disease/ procedure)]	Treatment (mg/day)	Follow-up	Primary endpoint	RRR (incidence of combined endpoint) [%]
Clopidogrel + aspirin vs aspirin	CHARISMA[88]	15 603 (CV)	Clopidogrel: 75 Aspirin: 75–162	Mean 28 mo	MI, stroke or CV death	6.8 (6.8 vs 7.3)*
	CHARISMA[89] (subgroup)	9478 (MI, stroke, PAD)	Clopidogrel: 75 Aspirin: 75–162	Mean 27.6 mo	CV death, MI or stroke	17 (7.3 vs 8.8)
	PCI-CURE[95]	12 562 (ACS)	Clopidogrel: 75 Aspirin: 75–325	12 mo	CV death, MI or stroke	20 (9.3 vs 11.4)
	CARESS[96]	100 (MES +)	Clopidogrel: 75, Aspirin: 75	7 d	MES (+)	39.8 (43.8 vs 72.7)
Clopidogrel + aspirin vs clopidogrel	MATCH[90]	7599 (high risk)	Clopidogrel: 75 Aspirin: 75	18 mo	Stroke, MI, vascular death	6.4 (15.7 vs 16.7)*
Aspirin + dipyridamole vs aspirin	ESPRIT[91]	2739 (TIA or stroke)	Dipyridamole: 400 Aspirin: 75	3.5 y	Death, nonfatal stroke, nonfatal MI, major bleeding	(13 vs 16)
Aspirin + dipyridamole vs aspirin/dipyridamole	ESPS-2[92]	6602 (TIA or stroke)	Aspirin: 50 Dipyridamole: 400	Mean 2 y	Stroke or death	(24.4 vs 13.2)[a] (24.4 vs 15.4)[a]
Clopidogrel + abciximab vs clopidogrel	ISAR-REACT 2[93]	2022 (PCI)	Clopidogrel: 600 Abciximab LD: 0.25 mg/ kg; MD: 0.125 μg/kg	30 d	Death, MI, revascularization	25 (8.9 vs 11.9)
Clopidogrel + aspirin vs ticlopidine + aspirin	Mueller et al.[94]	700 (CSI)	Clopidogrel: 50 Aspirin: 100 Ticlopidine: 500	18 mo	CV death	63 (2.3 vs 7.3)

a Relatively reduced rate.

ACS = acute coronary syndromes; **CSI** = coronary stent implantation; **CV** = cardiovascular disease; **LD** = loading dose; **MD** = maintenance dose; **MES** = microembolic signals; **MI** = myocardial infarction; **PAD** = peripheral arterial disease; **PCI** = percutaneous coronary interventions; **RRR** = relative risk reduction; **TIA** = transient ischaemic attack; + indicates positive; * $p > 0.05$.

Table V. Abbreviated and full names of trials investigating antiplatelet therapies

CAPRIE	Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events
CARESS	Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis
CHARISMA	Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance
DISPERSE-2	Dose ConfIrmation Study assessing anti-Platelet Effects of AZD6140 vs clopidogRel in non-ST-segment Elevation myocardial infarction – 2
ESPRIT	European/Australian Stroke Prevention in Reversible Ischaemia Trial
ESPS-2	European Stroke Prevention Study 2
ISAR-REACT 2	Intracoronary Stenting and Antithrombotic Regimen Rapid Early Action for Coronary Stenting 2
JUMBO-TIMI 26	Joint Utilization of Medications to Block Platelets Optimally – Thrombolysis In Myocardial Infarction 26
MATCH	Management of ATherosclerosis with Clopidogrel in High-Risk Patients
PCI-CURE	Percutaneous Coronary Intervention – Clopidogrel in Unstable Angina to Prevent Recurrent Ischaemic Events
PRINCIPLE-TIMI 44	PRasugrel IN Comparison to Clopidogrel for Inhibition of PLatelet Activation and AggrEgation – Thrombolysis In Myocardial Infarction 44
STAMI	Ticlopidine versus Aspirin after Myocardial Infarction
TACIP	Triflusal versus Aspirin in Cerebral Infarction Prevention
TAPIRSS	Triflusal versus Aspirin for Prevention of Infarction: Randomized Stroke Study
TARGET	Do Tirofiban And ReoPro Give similar Efficacy outcomes Trial
TRITON-TIMI 38	TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel – Thrombolysis In Myocardial Infarction 38

CHARISMA (see table V for full trial names) trial recently evaluated the value of dual antiplatelet therapy with clopidogrel plus aspirin.[87] Although no statistically significant benefit was found in the overall broad population of stable patients studied,[88] those with documented prior MI, ischaemic stroke or symptomatic peripheral arterial disease appeared to benefit from intensification of dual antiplatelet therapy beyond aspirin alone.[89] However, the addition of aspirin to clopidogrel in high-risk patients with a recent ischaemic stroke or transient ischaemic attack is associated with a nonsignificant reduction in major vascular events compared with patients treated with clopidogrel alone.[90] In contrast, the risk of life-threatening or major bleeding is increased following the addition of aspirin to clopidogrel. The ESPRIT study, combined with results of previous trials, provides sufficient evidence for the use of combination antiplatelet therapy of aspirin plus dipyridamole over aspirin alone in patients after cerebral ischaemia of arterial origin.[91,92] The ISAR-REACT 2 trial found that abciximab reduced the risk of adverse events in ACS patients without ST-segment elevation undergoing PCI after pretreatment with clopidogrel 600 mg;[93] however, subgroup analysis revealed that abciximab was beneficial only in those patients with elevated serum troponin levels. It is apparent that current clinical practice is to substitute clopidogrel for ticlopidine after stenting owing to the adverse effects of ticlopidine; however, Mueller et al.[94] found that ticlopidine combined with aspirin was associated with a significantly lower mortality than clopidogrel plus aspirin after the placement of coronary artery stents in unselected patients. Indeed, these discrepancies highlight the need for further long-term studies on combination therapy with antiplatelet agents.

3.3 Advances in Experimental Studies on Novel Antiplatelet Agents

There is an increasing number of novel antiplatelet agents being developed. Agents undergoing clinical investigation include E5555, SCH530348, YD-3 and regrelor (INS50589). Other agents under preclinical investigation include BX-667, JAQ-1, terutroban sodium (S18886) and YM-254890.

3.3.1 Novel Protease-Activated Receptor Antagonists

Thrombin is the most potent platelet agonist and plays a critical role in the development of arterial thrombosis. Human platelets express dual thrombin receptors, specifically protease-activated receptor (PAR) 1 and PAR4,[97] but there are still no therapeutic strategies that effectively target both receptor

subtypes. PAR1 and PAR4 appear to form a stable heterodimer that enables thrombin to act as a bivalent functional agonist. Targeting the PAR1–PAR4 complex may present a novel therapeutic opportunity to prevent arterial thrombosis.[98] Recent studies have identified that simultaneous antagonism of PAR1 and PAR4 is synergistic and provides more effective inhibition of thrombin-induced platelet activation than either PAR1 or PAR4 antagonism alone.[99]

There have been several peptide and nonpeptide antagonists of PAR1 developed, but only two PAR4 antagonists (YD-3 and YC-1). YD-3, as the first nonpeptide PAR4 antagonist, had little or no effect on thrombin-induced platelet aggregation alone and significantly enhanced the anti-aggregatory activity of PAR1 antagonists.[99] In human platelets, the inhibitory effect of YD-3 was significant only when the function of PAR1 was blocked or attenuated.[100] This may be one reason why YD-3 has not made it into clinical use. However, the results indicate that thrombin-induced platelet activation cannot be inhibited effectively by blocking just one of the thrombin receptor pathways and suggest a rationale for potential combination therapy in arterial thrombosis.[99] Although the two PAR4 antagonists are still under preclinical investigation, two of the orally administered PAR1 antagonists, namely E5555 and SCH530348, are currently undergoing evaluation in phase II studies. The efficacy and safety of the former is being evaluated in patients with CAD,[101] whereas the safety of various doses of the latter is being investigated in PCI.[44] As a selective antagonist of PAR1, RWJ-58259 has been shown to interfere with thrombotic activity in nonhuman primates, even in the presence of an active PAR4 receptor.[102] Perivascular application of RWJ-58259 *in vivo* significantly inhibited arterial injury-induced stenosis in a rat model of balloon angioplasty.[103] These preclinical results suggest potential clinical usefulness of RWJ-58259 in the treatment of thrombotic disorders and vascular injury associated with acute coronary interventions and atherosclerosis.

3.3.2 Novel $P2Y_{12}$ Receptor Antagonists

Irreversible platelet inhibitors, such as aspirin and clopidogrel, have limited antithrombotic efficacy in the clinic because of the risk of bleeding associated with their use. Regrelor (INS50589), a selective $P2Y_{12}$ receptor antagonist, is being developed for use where controlled, reversible modulation of platelet haemostatic function is needed. Phase I trials[104] have shown that regrelor is a well tolerated, reversible, competitive antagonist of ADP at the human platelet $P2Y_{12}$ receptor, with potential therapeutic usefulness in various cardiovascular settings. Preclinical data demonstrate that another reversible $P2Y_{12}$ receptor antagonist, namely BX-667, has a wider therapeutic index than clopidogrel in experimental models of thrombosis.[105]

3.3.3 Novel Thromboxane A_2/Prostaglandin H_2 Receptor Antagonists

It has already been shown that several TXA_2-receptor antagonists may be used in the treatment of cardiovascular diseases.[106] In addition, recent reports suggest that antagonism of TXA_2 receptors may be able to restrict vascular inflammation in atherosclerotic vessels.[107] In particular, terutroban sodium (S18886), a polysubstituted tetrahydronaphthalene derivative, is a new and highly selective TXA_2 receptor antagonist with a long duration of antiplatelet action.[108] It has been shown in two rabbit models that terutroban sodium inhibits the development of atherosclerosis.[109,110] Recently, in a porcine model of stent-induced thrombosis, it was found that blockade of the TXA_2 receptor by terutroban sodium provided a fast and potent platelet inhibitory effect, blocking both the ADP receptor and COX activation. In addition, the TXA_2 receptor antagonist had a more favourable bleeding risk profile.[111]

3.3.4 Novel Glycoprotein VI Antagonists

Collagen receptors offer attractive possibilities as alternative targets during the early stages of platelet activation. There are three major collagen receptors: (i) the $\alpha 2\beta 1$ integrin, responsible primarily for platelet adhesion to collagen; (ii) GP VI, the major signalling receptor for collagen; and (iii) GP Ib-V-IX, which acts as a collagen receptor indirectly via von Willebrand factor.[112] Several thrombosis models and experimental approaches suggest that all three are interesting targets and merit further investigation. GP VI may serve as the most attractive target for antithrombotic therapy because its inhibition or

absence results in profound protection against arterial thrombosis, but no major bleeding, in mice.[113] Moreover, direct targeting of GP VI provides significantly stronger protection against arterial thrombosis than soluble GP VI dimer.[114] JAQ-1, a monoclonal antibody against GP VI, has been shown to induce irreversible downregulation of the receptor and, consequently, long-term antithrombotic protection *in vivo*.[115] However, the use of JAQ-1 may result in defective haemostasis in patients with reduced $\alpha 2\beta 1$ levels or on concomitant aspirin therapy,[113] which limits its clinical use.

Recently, an anopheline antiplatelet protein (AAPP) isolated from the saliva of *Anopheles stephensi*, a human malaria vector mosquito, was found to have a strong and specific inhibitory effect against collagen-induced platelet aggregation.[116] The inhibitory mechanism involves direct binding of AAPP to collagen, which blocks platelet adhesion to collagen and inhibits the subsequent increase in intracellular Ca^{2+} concentrations. The binding of AAPP to collagen effectively blocked platelet adhesion via GP VI and integrin $\alpha 2\beta 1$. Moreover, intravenously administered recombinant AAPP strongly inhibited collagen-induced platelet aggregation *ex vivo* in rats.[116]

3.3.5 Novel Platelet G_q Antagonists

Recently, YM-254890, a novel inhibitor of the G_q signalling pathway, was found to inhibit ADP-mediated platelet aggregation by interfering with the guanosine diphosphate–guanosine triphosphate exchange pathway and blocking $P2Y_1$ receptor-mediated intracellular calcium mobilization.[117] YM-254890 has become a useful tool for the investigation of $G_{\alpha(q/11)}$-protein-coupled receptor signalling and the physiological roles of $G_{\alpha(q/11)}$.[118] Based on these findings, it has been suggested that this pharmacological inhibitor of the G_q signalling pathway has the potential to be developed as an antithrombotic drug. However, a G_q-specific approach would be required because inhibition of both G_q and G_{11} is likely to have deleterious effects in multiple organs.[119,120]

3.4 Summary

In summary, as indicated in tables II, III and IV and discussed in section 3, current antiplatelet therapies are based generally on specific signalling pathways in platelet activation, involve single agents acting on single targets and are mainly irreversible (see figure 3). However, many unresolved problems remain for both proven and experimental antiplatelet drugs, including their efficacy and safety, the patient populations in which their use is most appropriate, their optimal dosage, administration requirements, combination therapy, clinical evaluation, cost effectiveness and the 'resistance' phenomenon. In particular, aspirin resistance, with a reported prevalence of 24–40%,[121] and clopidogrel resistance (prevalence 6–58%)[66,122] have been investigated in some detail and, so far, the data indicate that resistance to these drugs may be attributed to factors such as compliance, dose, absorption, metabolism, drug interactions, genetic polymorphisms and the method used to define 'resistance' in the first place.[122] In fact, platelet aggregation should be normally distributed within populations,[123] as should aspirin and clopidogrel responsiveness. Antiplatelet resistance should be defined as a move away from the normal distribution of the platelet aggregation response.

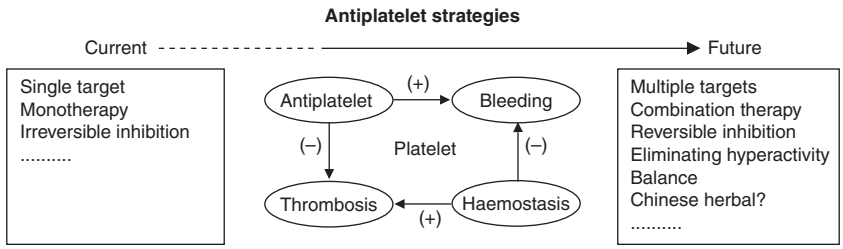

Fig. 3. Current and possible future strategies for the development of antiplatelet therapies. (+) indicates positive effect; (–) indicates negative effect.

4. Future Directions for Antiplatelet Therapy

Platelet activation is an integral component of the pathophysiology that leads to thrombotic and ischaemic diseases such as MI, cerebral stroke and peripheral arterial disease. Platelet inhibition is a major strategy to prevent arterial thrombosis, but is frequently associated with increased bleeding because of impaired primary haemostasis. Therefore, an ideal antiplatelet agent should specifically block thrombogenic platelet-dependent mechanisms in vascular diseases without interfering with normal platelet functions that are required in haemostasis and wound healing. In addition, these agents should be free of any major adverse effects. Although several antiplatelet strategies have been developed or are under preclinical or clinical investigation, none of the currently available antiplatelet drugs meets all these criteria. The current most widely used antiplatelet drugs (e.g. aspirin, ADP receptor antagonists, GP IIb/IIIa antagonists and PDE inhibitors) are relatively well tolerated by patients being treated for ischaemic diseases. However, the limited efficacy of these drugs in the setting of arterial thrombosis, their unfavourable adverse effect profiles, cost-to-benefit issues and the 'resistance' phenomenon substantiate the need for the development of newer and more efficacious antiplatelet and antithrombotic drugs.[122,124-126]

Therefore, future strategies for the development of new antiplatelet targets, drugs and treatment regimens is likely to take the following into consideration:

1. *Moving from a single target to multiple targets*. There are drugs that act at multiple sites within the platelet activation signalling pathway. For example, BM573, which blocks the TXA_2 receptor while inhibiting thromboxane synthase, inhibits arachidonic acid-induced platelet aggregation, prevents the formation of an occlusive thrombus and reduces the occurrence of MI without affecting heart rate or mean BP.[127] BM573 significantly decreased early atherogenesis and prevented progression of established atherosclerotic lesions.[128] Its dual thromboxane inhibitor action is effective in reducing atherogenesis in LDL-receptor-deficient mice.[128] In pigs, BM573 completely inhibited pulmonary hypertension and protected pigs from MI induced by coronary thrombosis.[129] In addition, the pharmacological compound TRA-418, which antagonizes the TXA_2 receptor and activates prostacyclin (IP) receptors, inhibits ADP-, PAR1-, activating peptide- and TXA_2-induced activation of GP IIb/IIIa, platelet aggregation and P-selectin expression in human platelets.[130] As such, TRA-418 may be more useful as an antiplatelet drug than either a TXA_2-receptor antagonist or a prostacyclin analogue alone.[130] Finally, the development of drugs with reversible action, such as the $P2Y_{12}$ receptor antagonist BX-667, which has a larger therapeutic index than clopidogrel in experimental models of thrombosis, may prove beneficial.[105]

2. *Moving from therapeutic regimens with a single drug to combination therapy*. Recent studies have shown that triple therapy (aspirin, clopidogrel and cilostazol) after coronary stent placement results in more potent inhibition of platelet aggregation induced by ADP and collagen compared with dual antiplatelet therapy (aspirin plus clopidogrel).[131] This suggests that triple therapy may be used clinically to prevent thrombotic complications after the placement of coronary stents. In particular, when aspirin treatment fails (clinical aspirin resistance), consideration should be given to replacing aspirin with another antiplatelet agent or combining aspirin therapy with two or more antiplatelet agents that inhibit other pathways of platelet activation and aggregation (e.g. an ADP receptor blocker, such as clopidogrel, or a PDE inhibitor, such as dipyridamole).[121]

3. *Discovery of new antiplatelet properties in other widely used drugs*. Because platelet activation is induced and enhanced by many factors, especially in certain pathological states, such as diabetes, hypertension and hyperlipidaemia, more and more antihypertensive, antilipidaemic and other drugs have been found to have direct or indirect antiplatelet effects, including nimodipine, verapamil, ACE inhibitors and HMG-CoA reductase inhibitors (statins).[132-135] However, some drugs may adversely interact with and undermine the effectiveness of antiplatelet agents (e.g. ibuprofen).[136] Finally, many herbs used in traditional Chinese medicine have been reported to inhibit platelet activation in humans.[137-140] These herbs have been widely used

for several thousands of years in traditional Chinese medicine to treat thrombotic and ischaemic diseases. Because herbs contain many active constituents, their antiplatelet and antithrombotic mechanisms are quite complicated and remain unclear. Moreover, the safety and efficacy of traditional herbs as antiplatelet agents need to be tested in large-scale randomized controlled trials.

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Correspondence: Dr *Yao-Zu Xiang*, Research Center of Tianjin University of Traditional Chinese Medicine, Nankai District, Anshanxi Road # 312, Tianjin, 300193, China.
E-mail: xiangyaozu@gmail.com