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REVIEW

$P2Y_{12}$ inhibitors: differences in properties and mechanisms of action and potential consequences for clinical use

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Currently, clopidogrel is recommended for treatment of patients with acute coronary syndrome and/or percutaneous coronary intervention. However, the delayed onset of the effect and the occurrence of poor platelet inhibition responders with clopidogrel as well as non-compliance to dual antiplatelet treatment are associated with a raised risk of stent thrombosis. The molecular target of the active metabolite of clopidogrel and several emerging antiplatelet treatments is the $P2Y_{12}$ receptor, which is the main platelet receptor responsible for ADP-induced platelet aggregation. Active metabolites of the thienopyridine prodrugs (ticlopidine, clopidogrel, and prasugrel) covalently bind to the $P2Y_{12}$ receptor and are irreversible, indirect platelet inhibitors. The newer, direct-acting $P2Y_{12}$ inhibitors (cangrelor and ticagrelor) change the conformation of the $P2Y_{12}$ receptor, resulting in reversible, concentration dependent inhibition of the receptor. An understanding of the similarities and differences in the properties and mechanisms of action of these new inhibitors compared with clopidogrel is needed in order to optimize the development and use of these agents in clinical practice. The objectives of this systematic review are to summarize the pharmacokinetics, pharmacodynamics, and pharmacogenetics of the different $P2Y_{12}$ inhibitors and to discuss the clinical implications for treatment of patients.

Keywords Thienopyridine • Purinoceptor $P2Y_{12}$ • Clopidogrel • Prasugrel • Cangrelor • Ticagrelor

Introduction

Platelet activation occurs at rupture of atherosclerotic plaques and at implantation of stent material in coronary arteries. The activation process involves the production of several platelet activation agonists including thrombin, thromboxane A2, and adenosine diphosphate (ADP), which amplify the platelet response and stimulate platelet aggregation. Adenosine diphosphate stimulates platelet activation through two G-protein coupled receptors, $P2Y_1$ and $P2Y_{12}$.[1] Although binding of ADP to both receptors is required for complete platelet aggregation, $P2Y_{12}$ is the predominant receptor involved in ADP-stimulated platelet activation of the glycoprotein (GP) IIb/IIIa receptor.[2] Binding of ADP to $P2Y_1$ stimulates activation of the GP IIb/IIIa receptor resulting in calcium mobilization, platelet shape change, and transient platelet aggregation.[3,4] Binding of ADP to $P2Y_{12}$ stimulates activation of the GP IIb/IIIa receptor resulting in enhanced platelet degranulation and thromboxane production, and prolonged platelet aggregation (*Figure 1*).[5–7]

The recommended platelet inhibitory treatment for patients with acute coronary syndrome (ACS) and in those undergoing percutaneous coronary intervention (PCI) with stent implantation is a combination of aspirin (acetylsalicylic acid) and $P2Y_{12}$ receptor inhibition with the thienopyridine, clopidogrel.[8,9] Despite the efficacy of this treatment on ischaemic events,[10–12] 15–40% of patients are poor responders to treatment, as evaluated by ADP-induced platelet aggregation.[13–17] Several trials show that such patients are at increased risk of stent thrombosis, myocardial infarction, and death.[18–21] Therefore, alternative antiplatelet treatments are being developed to overcome these limitations.

The thienopyridines (ticlopidine, clopidogrel, and prasugrel) are indirectly acting platelet inhibitors where the active metabolites of the thienopyridine prodrugs covalently and irreversibly bind to the $P2Y_{12}$ receptor during the entire lifespan of the platelet.[22,23] The newer, direct-acting $P2Y_{12}$ inhibitors (cangrelor and ticagrelor) change the conformation of the $P2Y_{12}$ receptor and, therefore, result in reversible inhibition of the receptor (*Figures 1* and *2*). The objectives of this systematic review are to summarize the

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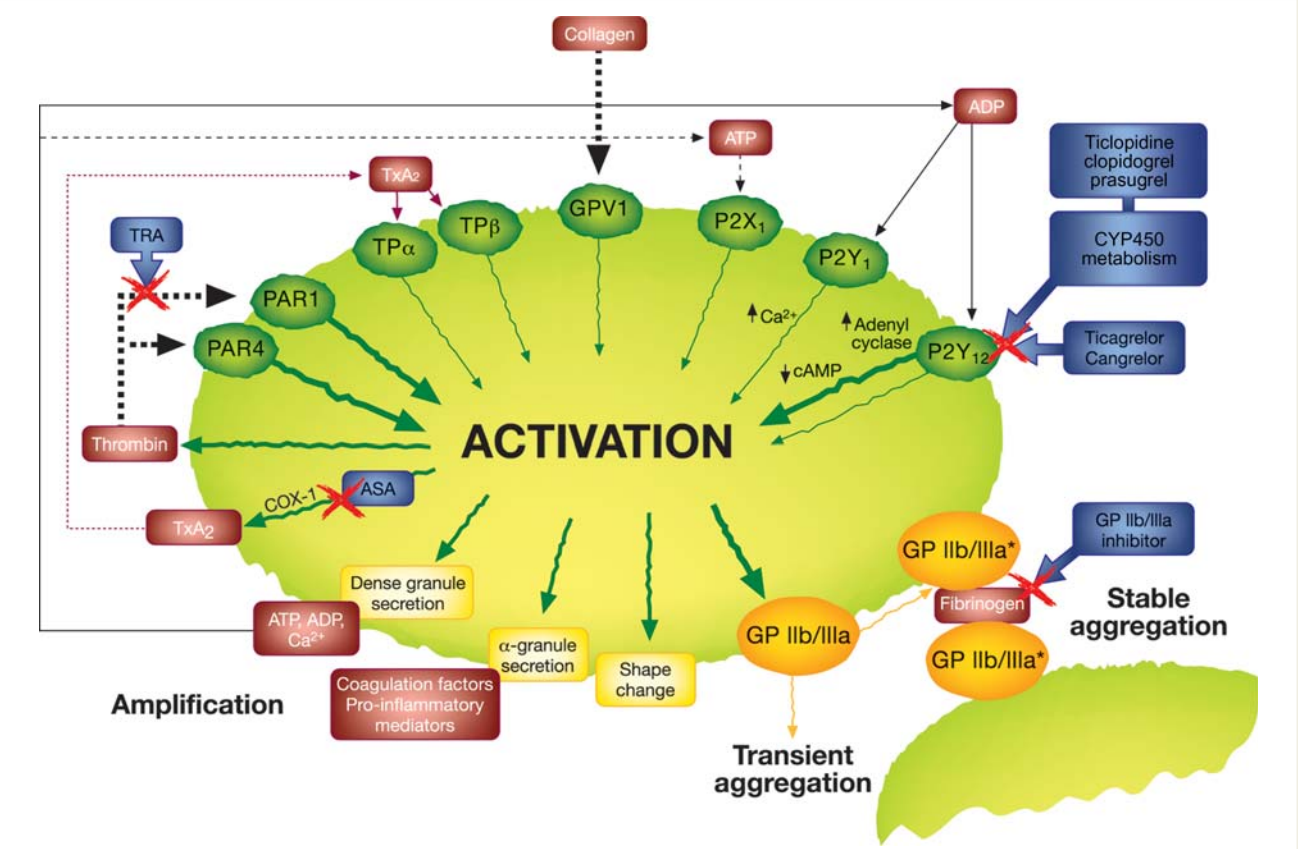

Figure 1 Platelet activation mechanisms (modified after Storey R.F. Biology and pharmacology of the platelet P2Y12 receptor. *Curr Pharm Des* 2006;**12:**1255–1259 with permission).

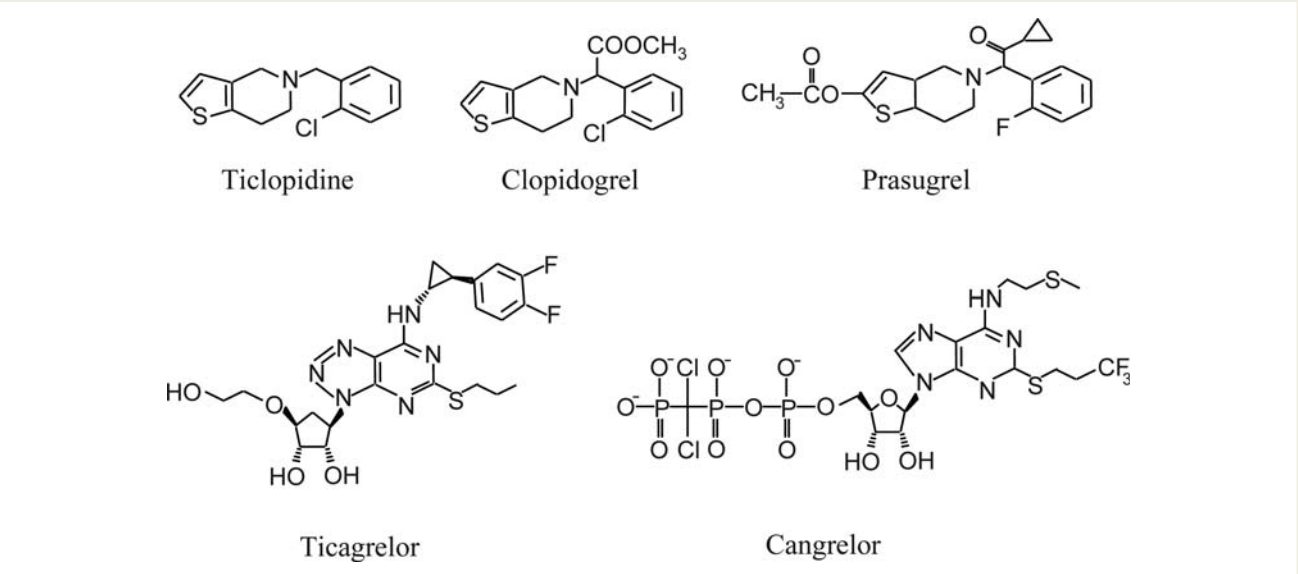

Figure 2 Chemical structures of the $P2Y_{12}$ inhibitors.

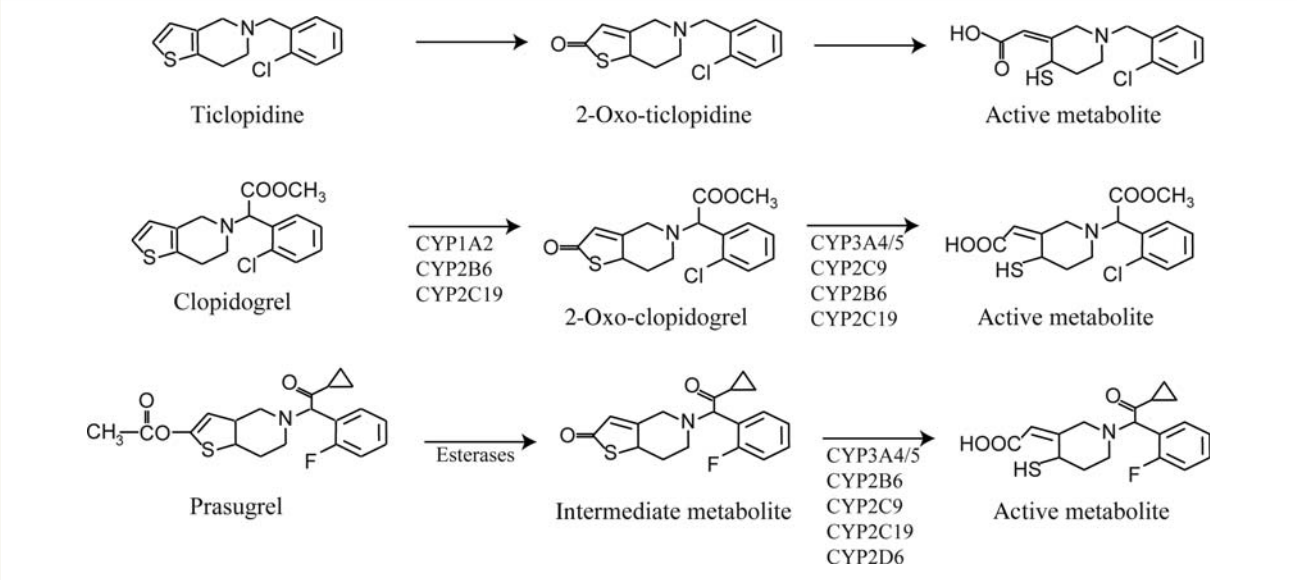

Figure 3 Major pathways leading to the formation of thienopyridine active metabolites.

pharmacokinetics, pharmacodynamics, and pharmacogenetics of the different $P2Y_{12}$ inhibitors and, within this context, to discuss the clinical implications for treatment of patients.

Methods

The databases searched were: MEDLINE via PubMed (1966 to January 2009), EMBASE (1988 to January 2009), and the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Abstracts of Reviews of Effects (1988 to January 2009). Search terms included: thienopyridine, ticlopidine, clopidogrel, prasugrel, AZD6140, ticagrelor, cangrelor, responder, low responder, non-responder, resistance, pharmacokinetics, pharmacodynamics, prodrug, active metabolite, pharmacogenetics, genetics, genetic polymorphism, cytochrome (CYP) P450, CYP2C9, CYP2C19, platelet aggregometry, light transmission aggregometry, LTA, vasodilator-stimulated phosphoprotein (VASP), VerifyNow, platelet inhibition, platelet aggregation, platelet reactivity index, ADP, $P2Y_{12}$. The search was limited to human studies published in English. The inclusion criteria were: all randomized controlled clinical trials, observational studies, and reviews investigating resistance to anti- $P2Y_{12}$ platelet therapy in patients and healthy subjects.

Pharmacology and pharmacokinetics of $P2Y_{12}$ inhibitors

Thienopyridine metabolism

Thienopyridines are metabolized in the liver and the intestines to active metabolites that covalently bind to the $P2Y_{12}$ receptor, causing irreversible platelet inhibition. Although the thienopyridines require CYP450 metabolism for the generation of active metabolites, the pathways leading to conversion to the active metabolites differ between the prodrugs (*Figure 3*). Ticlopidine is metabolized by at least five main pathways resulting in a minimum of 13, mostly inactive, metabolites.[24,25] Of these, one active metabolite, presumably formed via a CYP-dependent pathway, has been identified and shown to have antiplatelet activity.[25,26] Clopidogrel is metabolized by two pathways. One pathway converts most of a dose of clopidogrel to inactive metabolites by de-esterification.[27] The other pathway converts clopidogrel to its active metabolite by at least two CYP-dependent steps.[28,29] Of the several CYP enzymes identified, CYP1A2, CYP3A4/5, and CYP2C19 are considered to be the main contributors to active metabolite formation.[29–31] However, defective CYP2C19 and possibly also CYP2C9 and CYP2B6 genetic variants seem to be associated with decreased plasma concentrations (AUC and Cmax) of the active metabolite, lower platelet inhibition, and poor-responder status.[32–34] In contrast, prasugrel first undergoes rapid de-esterification to an intermediate thiolactone, which is then converted to the active metabolite in a single CYP-dependent step.[35–37] Pharmacokinetic and pharmacodynamic interaction studies indicate that the metabolism of prasugrel is not impacted by reduced function CYP polymorphisms.[31,32]

Thienopyridine pharmacokinetics

Thienopyridines are extensively and rapidly absorbed after administration. Unfortunately, the pharmacokinetics and the active metabolites of ticlopidine are not well investigated. Maximum plasma concentrations of ticlopidine are reached 1–3 h after a single oral dose (250 mg) and steady state concentrations are reached 3–5 days after repeated dosing (250 mg, twice daily).[25]

Maximal level of the clopidogrel active metabolite is reached ~1 h after dosing,[28,38] although the peak level is delayed at higher doses.[39] Plasma concentrations of clopidogrel metabolites increase in a dose-dependent, but less than dose-proportional, manner up to approximately a 600 mg dose of clopidogrel.[28,39,40] In general, maximum plasma concentrations of active

Table 1 Pharmacokinetics of current and emerging $P2Y_{12}$ inhibitors in humans

$P2Y_{12}$ inhibitor subjects	Dose	AUC (ng h/mL)	Cmax (ng/mL)	Tmax (h)	Apparent terminal half-life (h)	Apparent clearance (L/h)	Reference
Clopidogrel active metabolite							
Healthy	600 mg	126	38	1.4	1.0	NR	70
Healthy	300 mg	44	36	NR	NR	NR	43
Healthy	300 mg	185	141	NR	NR	NR	41
	600 mg	267	163	NR	NR	NR	
	300 mg load/75 mg daily, 7 days	66	64	NR	NR	NR	
	600 mg load/75 mg daily, 7 days	61	58	NR	NR	NR	
CAD	600 mg load	NR	NR	NR	NR	3420	39
	600 mg load/75 mg daily, 14 days	NR	NR	NR	NR	3420	
Prasugrel active metabolite							
Healthy	60 mg	534	512	NR	NR	NR	43
Healthy	60 mg	594	511	NR	NR	NR	41
	60 mg load/10 mg daily, 7 days	83	87	NR	NR	NR	41
CAD	60 mg	402	NR	NR	NR	149	39
	60 mg load/10 mg daily, 14 days	59	NR	NR	NR	149	
Ticlopidine parent compound							
NR	250 mg	NR	300	1–3	24–36	NA	25
	500 mg	NR	1900	1–3	24–36	NA	
	250 mg twice daily, 21 days	NR	900	1–3	24–40	NR	
Ticagrelor parent compound							
CAD	100 mg	3648	594	3.1	NR	NA	16
	100 mg twice daily, 14 days	5530	810	2.8	NR	21.6	
	100 mg twice daily, 28 days	5337	798	2.5	NR	22.6	
	200 mg	7581	1224	3.1	NR	NA	
	200 mg twice daily, 14 days	16 364	2278	2.6	NR	13.7	
	200 mg twice daily, 28 days	15 104	2200	2.7	NR	15.3	
	400 mg	NA	3374	2.0	NR	NA	
	400 mg twice daily, 14 days	31 723	3653	2.4	NR	15.0	
	400 mg twice daily, 28 days	31 338	3827	2.1	NR	15.6	
Ticagrelor active metabolite							
CAD	100 mg	899	135	3.7	NR	NR	16
	100 mg twice daily, 14 days	2108	261	3.0	NR	NR	
	100 mg twice daily, 28 days	1881	239	3.2	NR	NR	
	200 mg	1753	271	3.7	NR	NR	
	200 mg twice daily, 14 days	5448	654	3.3	NR	NR	
	200 mg twice daily, 28 days	5268	660	3.2	NR	NR	
	400 mg	NR	595	3.2	NR	NR	
	400 mg twice daily, 14 days	10 233	848	3.2	NR	NR	
	400 mg twice daily, 28 days	10 446	860	3.3	NR	NR	
Cangrelor							
ACS	Up to 4 μg/kg/min	NR	NR	NR	<5 min	44.3	47

ACS, acute coronary syndromes; AUC, area under the plasma concentration curve; CAD, coronary artery disease; Cmax, maximum plasma concentration; NA, not applicable; NR, not reported; Tmax, time to Cmax.

metabolite, up to 160 ng/mL with an AUC of 260 ng h/mL, are achieved after a 600 mg loading dose (*Table 1*).[41] No substantial increases in plasma concentrations of active metabolite are achieved with doses greater than 600 mg.[40] Approximately 40% of a 75 mg dose is excreted in urine and 35–60% is excreted in faeces.[38]

Maximal concentration of the active metabolite of prasugrel is reached within 0.5 h after dosing[14,31,39,42–46] In general, maximal plasma concentrations of 500 ng/mL of active metabolite with an AUC of 500 ng h/mL are achieved after a 60 mg loading dose (*Table 1*).[14,39,43] Plasma concentrations of prasugrel metabolites increase in a dose-dependent and dose-proportional manner up to loading dosages of 60–80 mg[41,42,44,47] with no accumulation of metabolites over 10 days of daily dosing.[45] Approximately 70% of a 15 mg dose is excreted in urine and 25% is excreted in faeces.[35]

Pharmacokinetics of direct-acting $P2Y_{12}$ inhibitors

Ticagrelor and cangrelor are high affinity ADP analogues that cause reversible inhibition of the $P2Y_{12}$ receptor (*Figure 2*). Both drugs directly antagonize ADP binding to the $P2Y_{12}$ receptor without the need for any metabolic activation. Cangrelor reaches steady state concentrations in plasma within 30 min of start of infusion (bolus 30 μg/kg and infusion 4 μg/kg/min). Cangrelor is rapidly cleared from plasma and has a very short half-life (less than 9 min in most patients).[48,49]

Ticagrelor is rapidly absorbed and undergoes enzymatic degradation after oral administration to at least one active metabolite, which has similar pharmacokinetics to the parent compound (*Table 1*).[15,16] Concentrations of ticagrelor and its active metabolite increase in plasma in a dose-dependent manner and are similar, irrespective of sex or age.[16] Maximum plasma concentrations and maximum platelet inhibition are reached 1–3 h after treatment. The plasma half-life is 6–13 h and accordingly the treatment is given twice daily. At steady state, exposure (AUC) to the active metabolite is ~35% of the exposure to the parent compound.

Pharmacodynamics of $P2Y_{12}$ inhibitors

In general, three methods are used to evaluate the pharmacodynamic response to $P2Y_{12}$ inhibitors: light transmittance aggregometry (LTA), the VerifyNow$^{\alpha}$ P2Y12 assay, and the VASP phosphorylation assay by flow cytometry.[2,50] With LTA *ex vivo* platelet rich plasma stimulated with ADP and inhibition of platelet aggregation (IPA) is calculated as the percent decrease in aggregation during treatment when compared with baseline.[19,51–53] The VerifyNow$^{\alpha}$ P2Y12 assay is a whole blood point-of-care light transmittance assay[54–56] and more specific for $P2Y_{12}$ inhibition as prostaglandin E1 is used to suppress the $P2Y_1$ receptor response[57] but still correlating well with LTA during clopidogrel[55,56,58] and prasugrel treatment.[58,59] Vasodilator-stimulated phosphoprotein phosphorylation measured by flow cytometry is also considered specific,[60] as addition of ADP to platelets in the presence of prostaglandin E1 does not lower VASP phosphorylation when the $P2Y_{12}$ receptor is inhibited.[58,61,62] Both in healthy subjects and coronary patients, there is a good agreement of platelet inhibition response to thienopyridines measured by ADP-induced LTA, the VerifyNow$^{\alpha}$ P2Y12 assay, and VASP phosphorylation.[58,59,62,63] Several studies have demonstrated that low response to clopidgrel at these measurements identify patients at risk of adverse clinical outcomes during treatment with clopidogrel.[18,51,64,65] However, currently there is limited information on the intra-individual variability in response to clopidogrel at repeated measurements with these assays over longer time periods.[66]

Clopidogrel pharmacodynamics

Significant platelet inhibition occurs within 1–2 h after a single loading dose of clopidogrel. The maximal level at an average of 30% platelet inhibition is achieved within 4–5 h after a 300 mg and is maintained for at least 24 h (*Table 2*).[13,27,43,67–71] This level of platelet inhibition is generally maintained until dosing is discontinued.[13,14,70,72,73] Platelet inhibition decreases to pre-treatment levels ~1 week after treatment is terminated.[28,72–74] Several studies using a variety of platelet function assays have shown that a poor response to clopidogrel occurs in a substantial proportion (15–40%) of individuals.[13,14,41,43,71,75–78] Patients who are poor responders to clopidogrel appear to have the similar response for the duration of their treatment.[21,79]

Platelet inhibition by clopidogrel is dose dependent, but not dose proportional, up to loading doses of 600 mg (*Table 2*).[27,39,80] Doubling the loading dose of clopidogrel from 300–600 mg results in reaching the maximal level of inhibition earlier, i.e. after 2–3 h, with an additional increase in average platelet inhibition of ~10–15% units.[40,67,68,77,81,82] Only limited further increase in inhibition is obtained by doses greater than 600 mg (*Table 2*).[39,40,67–69,81] Administration of loading doses greater than 300 mg or maintenance doses higher the 75 mg reduces the proportion of low responding patients, although there still remains variability in patient responses (*Table 2*).[83–87] Patients who are overweight have higher platelet reactivity than patients at normal weight and, therefore, can have a suboptimal response to clopidogrel therapy. Similarly, patients with type 2 diabetes mellitus have high platelet reactivity and a suboptimal response to standard clopidogrel treatment regimens.[78,88–90] Currently the CURRENT-OASIS-7 trial is comparing the 600 vs. 300 mg loading doses followed by 7 days of 150 vs. 75 mg daily maintenance dose on 30 day outcome in ACS patients managed with an early invasive strategy (www.clinicaltrials.gov NCT00335452).

Prasugrel pharmacodynamics

Platelet inhibition is observed 15–30 min after administration of the loading dose of 60 mg prasugrel and maximum 60–70% platelet inhibition is usually achieved within 2–4 h.[13,14,39,42–44] During maintenance treatment with 10 mg o.d., there is a steady state of at an average of 50% platelet inhibition.[13,14,41,44,45,47,91] After treatment is discontinued, platelet aggregation decreases to pre-treatment levels within 7–10 days.[42,44,45,47,91] Platelet inhibition is dose-dependent; with near maximum levels of inhibition (65–75%) occurring after administration of doses greater than 20–30 mg (*Table 2*).[13,47,91] Although dose-dependent increases in the concentration (AUC, Cmax) of active metabolite in plasma occur after administration of doses of up to 60 mg, corresponding augmentation of platelet inhibition by these higher doses has not been observed.[13,43,44,59] Administration of loading or maintenance doses of prasugrel results in a significantly more rapid onset, and more consistent and greater platelet inhibition, than administration

Table 2 Pharmacodynamics of current and emerging $P2Y_{12}$ inhibitors in humans

$P2Y_{12}$ inhibitor subjects (*n*)	Treatment regimen	Outcome (ADP, time of evaluation)	Reference
Clopidogrel			
Healthy ($n = 10$)	100 mg load	12% IPA (5 μM ADP, 2 h)	27
	200 mg load	31% IPA (5 μM ADP, 2 h)	
	400 mg load	39% IPA (5 μM ADP, 2 h)	
	600 mg load	42% IPA (5 μM ADP, 2 h)	
Healthy ($n = 10$)	600 mg load	51% IPA (20 μM ADP, 6 h)	70
Healthy ($n = 36$)	75 mg load + 75 mg daily	22%/48% IPA (5 μM ADP, 2–24 h/Day 5)	69
	150 mg load + 75 mg daily	21%/33% IPA (5 μM ADP, 2–24 h/Day 5)	
	225 mg load + 75 mg daily	35%/51% IPA (5 μM ADP, 2–24 h/Day 5)	
	300 mg load + 75 mg daily	31%/40% IPA (5 μM ADP, 2–24 h/Day 5)	
Healthy ($n = 24$)	25 mg daily	30% IPA (5 μM ADP, steady state)	72
	50 mg daily	46% IPA (5 μM ADP, steady state)	
	100 mg daily	53% IPA (5 μM ADP, steady state)	
	150 mg daily	73% IPA (5 μM ADP, steady state)	
CAD with aspirin ($n = 60$)	300 mg load	85% MPA (20 μM ADP, 4 h)	40
	600 mg load	70% MPA (20 μM ADP, 4 h)	
	900 mg load	65% MPA (20 μM ADP, 4 h)	
Non-ST-elevation ACS with coronary stenting and aspirin ($n = 292$)	300 mg load	61% MPA (10 μM ADP, >12 h)	66
	600 mg load	50% MPA (10 μM ADP, >12 h)	
PCI with coronary stenting with aspirin ($n = 40$)	75 mg daily	64% MPA (20 μM ADP, Day 30)	83
	150 mg daily	52% MPA (20 μM ADP, Day 30)	
Coronary stenting with aspirin ($n = 96$)	300 mg load + 75 mg daily	80%/57% MPA (20 μM ADP, 2 h/Day 5)	21
Prasugrel			
Healthy ($n = 24$)	30 mg load	57% IPA (20 μM ADP, 2 h)	42
	75 mg load	84% IPA (20 μM ADP, 2 h)	
Healthy aspirin-free ($n = 18$)	2.5 mg daily	ND IPA (20 μM ADP, 4 h)	45
	10 mg daily	60–70% IPA (20 μM ADP, 4 h)	
Healthy aspirin-free ($n = 21$)	40 mg load + 7.5 mg daily	74%/37% IPA (20 μM ADP, ≤24 h/Day 14)	44
	60 mg load + 15 mg daily	65%/~60% IPA (20 μM ADP, ≤24 h, Day 22)	
Prasugrel vs. Clopidogrel			
Healthy aspirin-free ($n = 68$)	Prasugrel 60 mg load	79% IPA (20 μM ADP, 4 h)	43
	Clopidogrel 300 mg load	33% IPA (20 μM ADP, 4 h)	
Healthy aspirin-free ($n = 30$)	Prasugrel 5 mg daily	39% IPA (20 μM ADP, Day 10)	46
	Prasugrel 10 mg daily	58% IPA (20 μM ADP, Day 10)	
	Prasugrel 20 mg daily	68% IPA (20 μM ADP, Day 10)	
	Clopidogrel 75 mg daily	16% IPA (20 μM ADP, Day 10)	
Healthy with aspirin ($n = 45$)	Prasugrel 20 mg load/5 mg daily	40%/39% IPA (20 μM ADP, 24 h/Day 5)	46
	Prasugrel 30 mg load/7.5 mg daily	45%/42% IPA (20 μM ADP, 24 h/Day 5)	
	Prasugrel 40 mg load/10 mg daily	53%/47% IPA (20 μM ADP, 24 h/Day 5)	
	Prasugrel 60 mg load/15 mg daily	69%/66% IPA (20 μM ADP, 24 h/Day 5)	
	Clopidogrel 300 mg load/75 mg daily	38%/41% IPA (20 μM ADP, 24 h/Day 5)	
Healthy aspirin-free ($n = 41$)	Prasugrel 60 mg load/10 mg daily	~90%/78% IPA (20 μM ADP, <6 h/<Day 9)	41
	Clopidogrel 300 mg load/75 mg daily	~50%/56% IPA (20 μM ADP, <6 h/<Day 4)	
	Clopidogrel 600 mg load/75 mg daily	~70%/52% IPA (20 μM ADP, <6 h/<Day 4)	
Stable CAD with aspirin ($n = 110$)	Prasugrel 60 mg load/10 mg daily	31%/43% (20 μM ADP, 2 h/Day 29) 8%/25% PRI (20 μM ADP, 2 h/Day 29) 93%/73% IPA VerifyNow P2Y12	14,57
	Clopidogrel 600 mg load/75 mg daily	55%/54% MPA (20 μM ADP, 2 h/Day 29) 56%/51% PRI (20 μM ADP, 2 h/Day 29) 44%/43% IPA VerifyNow P2Y12	
Stable CAD with aspirin ($n = 101$)	Prasugrel 40 mg load/5 mg daily	61%/43% IPA (20 μM ADP, 4 h/Day 7)	13
	Prasugrel 40 mg load/7.5 mg daily	61%/51% IPA (20 μM ADP, 4 h/Day 7)	
	Prasugrel 60 mg load/10 mg daily	68%/62% IPA (20 μM ADP, 4 h/Day 7)	
	Prasugrel 60 mg load/15 mg daily	68%/71% IPA (20 μM ADP, 4 h/Day 7)	
	Clopidogrel 300 mg load/75 mg daily	30%/40% IPA (20 μM ADP, 4 h/Day 7)	

Continued

Table 2 *Continued*

$P2Y_{12}$ inhibitor subjects (*n*)	Treatment regimen	Outcome (ADP, time of evaluation)	Reference
PCI with aspirin ($n = 201$)	Prasugrel 60 mg load/10 mg daily	75%/61% IPA (20 μM ADP, 6 h/Day 14) 90%/83% IPA VerifyNow$^{\alpha}$ P2Y12	17
	Clopidogrel 300 mg load/150 mg daily	32%/46% IPA (20 μM ADP, 6 h/Day 14) 51%/65% IPA VerifyNow$^{\alpha}$ P2Y12	
Ticlopidine			
Healthy ($n = 3$)	250 mg twice daily	43% IPA (5 μM ADP, steady state)	72
Ticagrelor			
Artherosclerosis with aspirin ($n = 200$)	Ticagrelor 100 or 200 mg twice daily Clopidogrel 75 mg daily	~70% IPA (20 μM ADP, steady state) ~40% IPA (20 μM ADP, steady state)	16
Non-ST-elevation ACS ($n = 91$)	Ticagrelor 90 mg twice daily Ticagrelor 180 mg twice daily Clopidogrel 75 mg daily	79% IPA (20 μM ADP, 4 weeks) 95% IPA (20 μM ADP, 4 weeks) 64% IPA (20 μM ADP, 4 weeks)	15
Cangrelor			
ACS with aspirin ($n = 39$)	2 or 4 μg/kg/min	>90% IPA WB impedance (3 μM ADP, 24 h)	47
PCI with aspirin ($n = 200$)	1, 2, or 4 μg/kg/min	87−99% IPA WB impedance (3 μM ADP, steady state)	100

ACS, acute coronary syndrome; ADP, adenosine diphosphate; CAD, coronary artery disease; IPA, inhibition of platelet aggregation by light transmittance aggregometry unless otherwise stated; MPA, maximal platelet aggregation; ND, not different from placebo; PCI, percutaneous coronary intervention; PRI, platelet reactivity index (vasodilator-stimulated phosphoprotein assay); WB, whole blood.

of clopidogrel in healthy subjects and in patients with coronary artery disease (*Table 2*).[13,14,17,41,43,47,58,91–93] In addition, subjects who are poor responders to clopidogrel respond adequately to prasugrel.[41,43] Accordingly, changing from clopidogrel therapy to prasugrel maintenance therapy, with or without a loading dose, results in further reductions in maximal ADP-induced platelet aggregation early after switching.[17,41]

Comparison between clopidogrel and prasugrel pharmacodynamics

The differences in the pharmacodynamics of clopidogrel and prasugrel are associated with the earlier production and greater concentration of the active metabolite of prasugrel in plasma compared with the equipotent active metabolite of clopidogrel.[14,41,43] Although there is a linear correlation between exposure to the clopidogrel active metabolite and platelet inhibition, the increase in platelet inhibition after administration of clopidogrel is not proportional to the increase in dose, particularly at doses greater than 300 mg, maybe dependent on an increased proportion being de-esterified to the inactive metabolite.[39–41,71] Subjects who are poor responders to clopidogrel, and have low platelet inhibition, have lower exposure to the active metabolite of clopidogrel than subjects who are normal responders.[14,39,43,78] Furthermore, *ex vivo* addition of the active metabolite of clopidogrel to blood samples in patients treated with a 600 mg loading dose and 75 mg maintenance dose provides additional and maximal platelet inhibition. Exposure to the active metabolite of a standard loading dose of prasugrel (60 mg) is significantly greater in magnitude than the exposure to the equipotent active metabolite of after dosing with either 300 or 600 mg clopidogrel.[32,41,43] Taken together, these findings suggest that poor responsiveness to clopidogrel is related to low concentration of and poor platelet exposure to the active metabolite in plasma rather than low sensitivity of the platelet P2Y12 receptor.[14]

Ticlopidine pharmacodynamics

When compared with the other thienopyridines, relatively little published data are available on the pharmacodynamics of ticlopidine. Platelet inhibition is dose-dependent, but little is known of relations between plasma concentrations of active metabolites and the degree of platelet inhibition.[25,94] Maximum platelet inhibition occurs 3–4 days after daily dosing in healthy subjects.[94,95] Recovery of platelet function occurs 3–4 days after discontinuation of 250 mg daily doses and 11–13 days after repeated 500 mg doses. Combination ticlopidine and aspirin enhances platelet inhibition above the level of either drug alone.[96,97]

Direct-acting $P2Y_{12}$ inhibitor pharmacodynamics

Ticagrelor results in an average of 50–60% inhibition of ADP-induced maximal platelet aggregation 2–4 h after a 180 mg loading dose, and this level of inhibition is sustained during maintenance therapy with 90 mg b.i.d.[15,16] Although the plasma concentration of ticagrelor is dose-dependent, the increase in platelet inhibition by increases in doses above 90 mg b.i.d. is relatively small. When compared with clopidogrel, ticagrelor provides earlier onset and more consistent and more pronounced platelet aggregation (*Table 2*). In patients with stable artherosclerosis, ticagrelor in doses of 100 mg b.i.d. or higher resulted in ~90% inhibition of final extent of ADP-induced platelet aggregation compared with ~60% with clopidogrel 75 mg daily.[16] In patients with non-ST-segment elevation ACS, ticagrelor provided further

IPA in patients previously treated with clopidogrel, irrespective of the level of patient responsiveness to clopidogrel before switching.[15] Accordingly there are very few low responders to ticagrelor treatment.

Cangrelor i.v. has a rapid onset of its platelet inhibitory effect with maximal inhibition within 15 min and a rapid reversal after treatment is discontinuation (*Table 2*). Steady state platelet inhibition is reached within 30 min after infusion starts and returns to pre-treatment levels in most patients within an hour after cessation of treatment.[48,98] There are some *in vitro* and healthy volunteer data indicating that cangrelor may competitively inhibit the antiplatelet effects of thienopyridine active metabolites.[99,100]

Pharmacogenetics of $P2Y_{12}$ inhibitors

Part of the variability in the individual response to platelet inhibitory agents may be due to differences in genetics. To date, genetic variations in several genes involved in CYP450 metabolism and in the expression of platelet receptors have been proposed to explain part of the variability in clopidogrel responsiveness between individuals. However, also variations in absorption and receptor reactivity might contribute to the variability within and between individuals.

Cytochrome P450 enzymes

The interindividual variability in clopidogrel responsiveness may be a result of functional variations in genes encoding at least two of the hepatic CYP450 enzymes involved in active metabolite formation (*Table 3*). CYP3A4, CYP3A5, and CYP2C19 comprise the most abundant hepatic P450 enzymes[101] and are considered to be the main enzymes involved in thienopyridine metabolism.[30,31,101–106] Although CYP3A4 and CYP3A5 are highly polymorphic no genetic variants that affect clopidogrel pharmacokinetics or responsiveness yet been identified.[32,103,107–109]

Combined data from several studies suggest that defective CYP2C19 activity is responsible for some of the variability in clopidogrel responsiveness among patients.[32–34,103,107,110–112] The CYPC19*2 mutant allele, which is a non-functional variant of CYP2C19,[113] is associated with higher platelet reactivity compared with functional CYP2C19 in healthy subjects receiving either loading and/or maintenance doses of clopidogrel.[32,107,110,114] In addition, the pharmacokinetic profile of clopidogrel differs between individuals with and without CYPC19*2. Two studies have shown that after a loading dose of clopidogrel, healthy subjects who carry CYPC19*2 have: (i) higher plasma concentrations of clopidogrel (AUC and Cmax)[110] and lower concentrations of the active metabolite of clopidogrel (AUC, Cmax)[32] than subjects who carry a functional CYP2C19 allele. These findings are supported by recent studies showing that the presence of CYPC19*2, compared with functional CYPC19, is associated with higher platelet reactivity and aggregation in patients treated with loading and maintenance doses of clopidogrel.[33,86,103,104] Although the genetic variants of CYP2C19 are associated with decreased plasma concentrations (AUC and Cmax) of the active metabolite of clopidogrel, lower inhibition of platelet reactivity, and poor-responder status, it has no effect on the pharmacokinetics and pharmacodynamics of prasugrel.[32,33] Recently, several CYPC19 loss-of-function alleles have been linked to recurrent thrombotic coronary events, such as myocardial infarction and stent thrombosis in patients with acute coronary disease treated with clopidogrel.[34,111,112] Also, the presence of two variant alleles of the *ABCB1* gene, a gene modulating clopidogrel absorption, has been shown to increase the risk of death from any cause, non-fatal stroke, or myocardial infarction.[111] It is noteworthy that the CYP2C19 defective genotypes, such as CYPC19*2 particularly, are common with frequencies ranging from 20 to 30% in Caucasians, 30 to 45% in African-Americans, and up to 50 to 65% in East Asians,[115–117] suggesting differences in clinical efficacy of clopidogrel at different ethnic background.

Cytochrome P450 and proton pump inhibitors

All proton pump inhibitors (PPIs), except for rabeprazole and pantoprazole, are extensively metabolized by the hepatic CYP450 enzyme, CYPC19, and to a lesser extent, CYP3A4(118) and, therefore, may interact with the metabolism of thienopyridines. Omeprazole is considered to have a higher potential for drug–drug interactions than other PPIs because of its ability to inhibit CYP2C19 activity.[118] In patients undergoing PCI, co-administration of omeprazole with dual antiplatelet therapy has been associated with higher platelet reactivity (measured using the VASP assay) compared with patients who did not receive omeprazole.[119] Also, lansoprazole has been shown to cause a small reduction in platelet inhibition 24 h after clopidogrel dosing.[120,121] More recently, the hypothesis that PPIs interact with clopidogrel metabolism was assessed in a population-based, nested case–control study comprising patients treated with clopidogrel after an acute myocardial infarction.[122] In this study, the current use of a PPI was associated with increased risk of re-infarction (odds ratio 1.27, 95% confidence interval 1.03–1.57).

Polymorphism of platelet receptors

Several genetic variants of both the $P2Y_1$ and $P2Y_{12}$ receptor genes have been implicated in the variation in platelet reactivity to ADP in healthy subjects.[123,124] Of the two $P2Y_{12}$ haplotypes (H1 and H2) identified, the minor H2 variant is associated with higher than wild-type platelet reactivity and a greater risk of atherosclerosis.[123,125] To date, no variants of either receptor have been found to be associated with clopidogrel responsiveness or platelet reactivity after treatment of patients with low or high loading doses of clopidogrel (*Table 3*).[104,108,126–129]

Conclusions and implications

The delayed onset and variability in platelet inhibition[13–17,78] with clopidogrel is associated with an increased risk of stent thrombosis and ischaemic events in poorly responsive patients.[18–21,130,131] The limited information on the appropriate target level and the variability of current platelet assays make them still not recommendable for tailoring of dosing in routine care.[51,54,55,58,62,78,132] In PCI-treated ACS patients, prasugrel provides a better protection

Table 3 Pharmacogenetics of $P2Y_{12}$ inhibition during treatment with clopidogrel in humans

Gene variant	Subjects (*n*)	Clopidogrel regimen	Variant compared with wild-type	Reference
CYP3A4/5				
CYP3A4*1B CYP3A5*3	Non-ST-elevation ACS ($n = 603$)	600 mg load dose	ADP-induced platelet aggregation[a] VASP phosphorylation[a] P-selectin surface expression[a]	105
CYP3A5*3	PCI ($n = 54$)	300 mg load/75 mg daily 600 mg load	ADP-induced platelet aggregation[a] Whole blood aggregometry[a] P-selectin surface expression[a]	110
IVS10+12A	Heterogenous ACS ($n = 1419$)	600 mg load/75 mg daily	ADP-induced platelet aggregation[a]	106
IVS10+12A	Stable CAD ($n = 82$)	300 mg load/75 mg daily	ADP-induced platelet aggregation[a] GP IIb/IIIa surface expression[b] Clopidogrel responder status[c]	104
IVS10 + 12A	Healthy ($n = 97$)	300 mg load/75 mg daily	ADP-induced platelet aggregation[a]	108
CYP3A5 variants	Healthy ($n = 89$)	300 mg load	ADP-induced platelet aggregation[a] Active metabolite pharmacokinetics[a]	32
CYP3A5*3	Healthy ($n = 29$)	75 mg daily	ADP-induced platelet aggregation[a] VASP phosphorylation[a] Clopidogrel responder status[a]	109
CYP3A5*3	Healthy ($n = 22$)	300 mg load/75 mg daily	ADP-induced platelet aggregation[a] Active metabolite Cmax, AUC[a]	111
CYP2C19				
CYP2C19*2	Non-ST-elevation ACS ($n = 603$)	600 mg load	ADP-induced platelet aggregation[d] VASP phosphorylation[d] P-selectin surface expression[d]	105
CYP2C19*2	CAD ($n = 55$)	600 mg load 600 mg load/75 mg daily	ADP-induced platelet aggregation[d] VASP phosphorylation[d] Clopidogrel responder status[c] Active metabolite Cmax, AUC[b]	33
CYP2C19*2	Heterogenous ACS ($n = 81$)	150 mg daily	Clopidogrel responder status[a]	85
CYP2C19*2	Heterogenous ACS ($n = 1419$)	600 mg load/75 mg daily	ADP-induced platelet aggregation[d]	106
CYP2C19*2	Healthy ($n = 47$)	300 mg load	ADP-induced platelet aggregation[d] VASP phosphorylation[d] Active metabolite Cmax, AUC[b]	116
CYP2C19*2	Healthy ($n = 97$)	300 mg load/75 mg daily	ADP-induced platelet aggregation[d]	108
CYP2C19*2	Healthy ($n = 29$)	75 mg daily	ADP-induced platelet aggregation[d] VASP phosphorylation[d] Clopidogrel responder status[c]	109
CYP2C19*2	Healthy ($n = 89$)	300 mg load	ADP-induced platelet aggregation[d] Active metabolite Cmax, AUC[b] Clopidogrel responder status[c]	32
CYP2C19*2	Healthy ($n = 24$)	300 mg load/75 mg daily	ADP-induced platelet aggregation[d] Clopidogrel Cmax, AUC[d] Clopidogrel responder status[c]	112
CYP2C9				
CYP2C9 variants	Healthy ($n = 89$)	300 mg load	ADP-induced platelet aggregation[d] Active metabolite Cmax, AUC[b]	32
$P2Y_{12}$ receptor H1/H2 haplotype				
H2 haplotype	PCI ($n = 54$)	300 mg load/75 mg daily 600 mg load	ADP-induced platelet aggregation[a] Whole blood aggregometry[a] P-selectin surface expression[a]	110
H2 haplotype (T744C)	Heterogenous ACS ($n = 1419$)	600 mg load/75 mg daily	ADP-induced platelet aggregation[a]	106
H2 haplotype (T744C)	PCI ($n = 120$)	300 mg load	ADP-induced platelet aggregation[a] GP IIa/IIIb surface expression[a] P-selectin surface expression[a] Clopidogrel responder status[a]	129

Continued

Table 3 *Continued*

Gene variant	Subjects (*n*)	Clopidogrel regimen	Variant compared with wild-type	Reference
H2 haplotype (T744C)	Non-ST-elevation ACS ($n = 597$)	600 mg load	ADP-induced platelet aggregation[a] VASP phosphorylation[a] P-selectin surface expression[a] Clopidogrel responder status[a]	128
H2 haplotype	CAD prior to stenting ($n = 416$)	600 mg clopidogrel	ADP-induced platelet aggregation[a]	127
H2 haplotype (T744C)	CAD ($n = 119$)	300 mg load or 75 mg daily	ADP-induced platelet aggregation[a] GP IIa/IIIb surface expression[a] P-selectin surface expression[a]	126
$P2Y_1$ receptor				
1622A>G	PCI ($n = 120$)	300 mg load	ADP-induced platelet aggregation[a] GP IIa/IIIb surface expression[a] P-selectin surface expression[a] Clopidogrel responder status[a]	129

ACS, acute coronary syndrome; ADP, adenosine diphosphate; AUC, area under the plasma concentration curve; CAD, coronary artery disease; Cmax, maximum plasma concentration; PCI, percutaneous coronary intervention; VASP, vasodilator-stimulated phosphoprotein assay.
[a]No difference between variant and wild-type or no association.
[b]Lower effect with variant compared with non-variant or wild-type.
[c]Association with responder status.
[d]Higher effect with variant compared with non-variant or wild-type.

against ischaemic events but with a raised risk of major bleeding.[133] The higher efficacy of prasugrel is related to its simpler metabolism, more rapid conversion to the active metabolite, and the lack of influence of genetic variability. The more rapid onset and offset of platelet inhibition by the directly acting and reversible P2Y12 inhibitors may provide further advantages as recently indicated in press releases concerning the primary outcome of 6–12 months treatment with oral ticagrelor in the PLATO trial (www.clinicaltrials.gov NCT00391872) although not achieved with 2 h intravenous infusions with cangrelor[48,98] in the CHAMPION trials (www.clinicaltrials.gov NCT00305162, NCT00385138).

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CARDIOVASCULAR FLASHLIGHT

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Contrast-enhancing left ventricular apical thrombus

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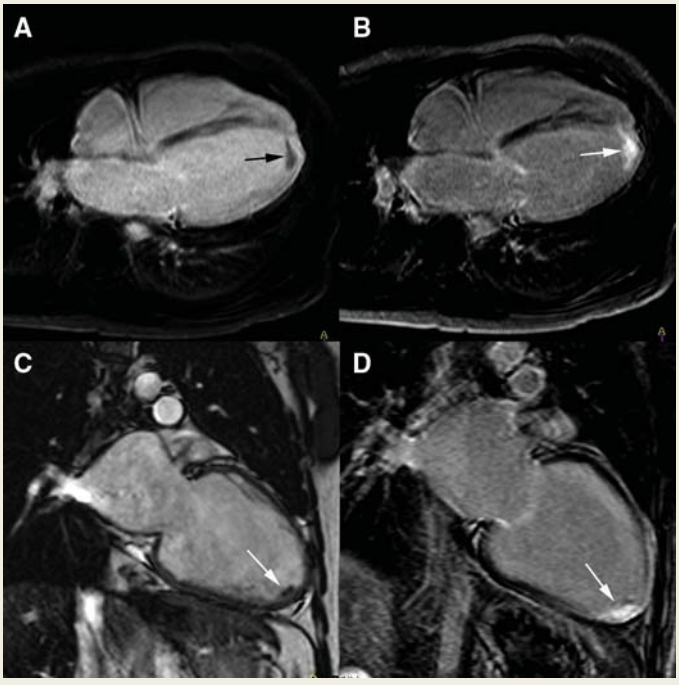

A 27-year-old man was transferred to our hospital because of heart failure. He had been diagnosed with dilated cardiomyopathy 6 months ago. Since at initial echocardiography a left ventricular (LV) apical thrombus (size 3 cm) was shown, the patient was treated with oral anticoagulation therapy. To exclude non-compaction cardiomyopathy of the LV, he was referred to cardiac magnetic resonance (CMR). Cine CMR showed a severely dilated and dysfunctional LV (EDV: 329 mL, EF: 15%) with severe diffuse hypokinesia. The LV showed a normal trabecular pattern, without evidence of non-compaction cardiomyopathy. The LV apical mural thrombus was still present but had considerably decreased in volume ($19 \times 14 \times 5$ mm) (*Panel C*, white arrow). Contrast-enhanced magnetic resonance imaging (MRI) demonstrated myocardial enhancement subepicardially in the LV inferolateral and apicolateral wall and midseptum. The patterns of myocardial enhancement and the normal coronary patency on coronary angiography were suggestive of a previous history of myocarditis. Remarkably, the apical LV thrombus showing a typical hypointense appearance on the early images acquired post-contrast administration (*Panel A*, black arrow) became strongly hyperintense on the late images acquired more than 10 min after contrast administration (*Panels B and D*, white arrow).

Contrast-enhanced MRI has shown extremely useful in the diagnosis and characterization of myocardial diseases. An added bonus of this technique is the enhancement of the blood pool, having a bright (or hyperintense) appearance, thus allowing to accurately detect abnormal intraluminal structures, such as cardiac thrombi. Typically, intracardiac thrombi remain hypointense after contrast injection. But, it is known from histopathology that chronic thrombi may be vascularized, although imaging data about vascularized thrombi are sparse. This case nicely shows the progressive, strong enhancement of the apical LV thrombus on consecutive post-contrast imaging. Although most thrombi encountered on contrast-enhanced MRI, in daily routine, remain dark, one should be familiar with these atypical presentations. This may be relevant when there is a need to differentiate thrombi from other masses such as tumours. Although we do not have histological proof that the LV apical mass was a thrombus; an intracavitary mass that shrinks under anticoagulation therapy in a patient with severely dilated cardiomyopathy virtually confirms the diagnosis of a thrombus. An unresolved issue is whether the enhancement occurs due to contrast diffusion from the LV cavity or due to thrombus vascularization.