

The use of amlodipine, but not of P-glycoprotein inhibiting calcium channel blockers is associated with clopidogrel poor-response

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Summary

Clopidogrel is a prodrug that has to be converted *in vivo* to its active metabolite by cytochrome (CYP)P450 iso-enzymes. As calcium channel blockers (CCBs) are inhibitors of CYP3A4, concomitant use of these drugs might play a role in the wide inter-individual variability in the response to clopidogrel. However, some CCBs also have strong inhibitory effects on the drug transporter P-glycoprotein (Pgp), which mediates clopidogrel's intestinal absorption. It was the aim of this study to evaluate the effect of co-administration of Pgp-inhibiting and non-Pgp-inhibiting CCBs on on-clopidogrel platelet reactivity in patients on dual antiplatelet therapy undergoing elective percutaneous coronary intervention (PCI). In a total of 623 consecutive patients undergoing elective PCI treated with clopidogrel and aspirin, platelet reactivity to 5 and 20 μ M adenosine diphosphate (ADP) and clopidogrel poor-response (defined as > 70% platelet aggregation to 20 μ M ADP) were evaluated by light transmittance aggregometry. A total of 222 patients (35.6%) were on CCB treatment, of which 98 used Pgp-inhibiting CCBs (verapamil, ni-

fedipine, diltiazem, barnidipine) and 124 patients used the non-Pgp-inhibiting CCB amlodipine. Adjusted mean ADP-induced on-clopidogrel platelet reactivity was significantly higher in both users of Pgp-inhibiting CCBs and amlodipine as compared to CCB non-users (all $p < 0.05$). However, only the use of amlodipine was significantly associated with a 2.3-fold increased risk of clopidogrel poor-response. This study demonstrates that concomitant use of Pgp-inhibiting CCBs and amlodipine increases on-clopidogrel platelet reactivity. Only amlodipine was associated with clopidogrel poor-response. The drug-drug interaction between clopidogrel and amlodipine might be more clinically relevant as compared to P-glycoprotein-inhibiting CCBs.

Keywords

Clopidogrel, drug-drug interaction, percutaneous coronary intervention, platelet reactivity, calcium channel blockers, P-glycoprotein, CYP3A4

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Introduction

Dual antiplatelet therapy with clopidogrel and aspirin has become standard treatment after percutaneous coronary interventions (PCI) (1). The intestinal efflux of clopidogrel is dependent on P-glycoprotein (Pgp). Pgp-mediated efflux reduces the intracellular accumulation of clopidogrel, thereby diminishing its efficacy (2). Clopidogrel is a prodrug that needs to be converted *in vivo* to generate its active metabolite. Conversion into the active compound occurs in a two-step process, in which the hepatic cytochrome (CYP) P450 iso-enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4,5 are involved (3). The active thiol meta-

bolite irreversibly inhibits the adenosine diphosphate (ADP) P2Y₁₂ receptor on the platelets surface (4, 5). Platelet response to clopidogrel is highly variable between individuals (6). The activity of the CYP-enzymes and overexpression of Pgp are thought to influence the antiplatelet effect of clopidogrel. Genetic variants of genes encoding CYP iso-enzymes and Pgp are associated with diminished platelet inhibition during clopidogrel treatment and some with an increased risk of atherothrombotic events (3, 7–16). Drugs that are substrates or inhibitors of the same CYP iso-enzymes or Pgp might also influence the antiplatelet effect of clopidogrel. Calcium channel blockers (CCBs) have been used for many years to treat angina pectoris, hypertension and other car-

diovascular diseases (17). Two recent studies suggested that CCBs reduce the pharmacodynamic response to clopidogrel and increase the risk of adverse atherothrombotic events by the inhibition of CYP3A4 (18, 19). However, within the class of CCBs, there are substantial pharmacokinetic differences. All CCBs are substrates and inhibitors of CYP3A4 (20). Importantly, some CCBs (nifedipine, nicardipine, barnidipine, felodipine, leteridipine, verapamil and diltiazem) also have strong inhibitory effects on Pgp activity ("Pgp-inhibiting CCBs") (20). Other CCBs like nimodipine, nisoldipine, isradipine and amlodipine exhibit no inhibitory effects on Pgp activity ("non-Pgp-inhibiting CCBs") (20). Due to these differences within the class of CCBs, different clinical relevance of drug interactions with clopidogrel are expected. Siller-Matula et al. and Gremmel et al. did not perform comparative analyses within the class of CCBs due to small sample size.

The aim of this study was to investigate the impact of co-administration of different CCBs on on-clopidogrel platelet reactivity in a large cohort of patients on dual antiplatelet therapy undergoing elective PCI.

Methods

Study design

In a prospective observational study we measured on-clopidogrel platelet reactivity in a large cohort of consecutive patients undergoing elective coronary stent implantation. All patients were on dual antiplatelet therapy with aspirin and clopidogrel at the time of inclusion. All patients received clopidogrel (75 mg) and aspirin (80 mg) daily for more than five days prior to the intervention. Exclusion criteria were: acute myocardial infarction with ST-segment elevation within 48 hours from symptom onset, allergies or contra-indications to heparin, increased risk of bleeding, malignancies, pregnancy or haematological disorders including thrombocytopenia and treatment with GP IIb/IIIa inhibitors during the 14 days before platelet function testing. Information on the use of CCBs and other co-medication was obtained from community pharmacies. A CCB-user was defined as a subject who was on CCB treatment for ≥ 7 days prior to the coronary stent implantation. The study protocol was approved by the hospital's Medical Ethics Committee, and informed consent was obtained from each patient.

Blood sampling

Prior to PCI and before heparinisation, blood was drawn from the femoral arterial sheath in 3.2% citrate tubes for platelet function testing. The first 10 ml of free-flowing blood were discarded.

Platelet function assays

The magnitude of on-clopidogrel platelet reactivity was assessed by light transmission aggregometry (LTA) using the ATRACT 4004 four-channel light transmission aggregometer (LABiTec, Ahrensburg, Germany). Samples were centrifuged for 10 min at 150 g to obtain native platelet rich plasma (PRP). Maximal platelet aggregation (defined as the maximum extent of platelet aggregation achieved in any time during the run of 10 minutes) was quantified in non-adjusted PRP after stimulation with 5 and 20 μM ADP. The magnitude of on-clopidogrel platelet reactivity in whole blood, expressed as P2Y12 Reaction Units (PRU), was measured with the VerifyNow P2Y12 Point-of-Care test cartridge system (Accumetrics, San Diego, CA, USA), as described previously (21, 22). LTA is considered to be the gold standard for determining the effects of antiplatelet therapy on platelet function, but logistical demands make it difficult to use in daily practice. The VerifyNow P2Y12 assay[®] is a point-of-care platelet function assay which has the specific purpose to rapidly inform the clinician about the magnitude of platelet inhibition that is achieved with the individual antiplatelet regimen (23). All measurements were completed within 2 hours of blood collection.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviation (SD) or median with interquartile range [IQR]. Categorical variables were expressed as frequencies and percentages. For baseline characteristics, continuous data were analysed by analysis of variance (ANOVA) and categorical data by chi-square test when appropriate. Kolmogorov-Smirnov test was used to test for normal distribution of continuous data.

ANOVA and, in case of a significant result, LSD (least significant difference) post-hoc tests, were used to analyse mean differences in normally distributed on-clopidogrel platelet reactivity between treatment groups. Multivariate linear regression was used to adjust for confounding factors (gender, age, body mass index, diabetes mellitus, prior myocardial infarction, hypertension, current smoking, left ventricular ejection fraction [LVEF] $< 45\%$, duration of clopidogrel administration before the coronary stent implantation [in days] and the use of proton pump inhibitors). For determining the influence of concomitant use of the CCBs on the clopidogrel responder status, crude and adjusted odds ratios (ORs) with their 95% confidence interval (CI) were calculated using logistic regression analysis. A poor-responder was defined as a clopidogrel-treated subject with more than 70% aggregation using 20 μM ADP (LTA) or with VerifyNow P2Y12 PRU value of more than 235 (24, 25). Sample size calculation for the present study was based on results of the study of Siller-Matula et al. in which an approximately 25% relative increase of ADP-induced platelet aggregation was observed in the group of patients on concomitant CCB treatment (18). Under the assumption that approximately 35% of the patients is on concomitant CCB treatment, CCB treatment is as-

sociated with a 25% relative increase (from $52\% \pm 25$ to $65\% \pm 25$) of ADP-induced platelet aggregation and a power of 90% with a two-sided α -value of 0.05, a sample size of at least 60 patients in each CCB-treatment group and 180 non-users of CCBs (overall sample size of 300 patients) was required. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 15.0.1 for Windows; SPSS Chicago, IL, USA).

Results

Patient characteristics

A total of 623 consecutive patients who were on maintenance therapy with aspirin and clopidogrel were enrolled in this study. From the study population, 222 (35.6%) patients were on CCB treatment at the time of platelet function testing. Among them, 98 patients used Pgp-inhibiting CCBs (verapamil 320 mg [n=1], diltiazem [n=57, mean dose 213.4 ± 52.5 mg], barnidipine 10 mg [n=2] and nifedipine [n=38, 43.0 ± 15.2 mg]). The remaining 124 patients were treated with amlodipine (mean dose 5.5 ± 1.5 mg), which does not inhibit Pgp. The median duration time [IQR] of CCB treatment before platelet function testing was 48 days [294 days]. The baseline characteristics of the study population according to CCB treatment are shown in ► Table 1. In univariate analysis, significant differences between the groups regarding the variables age ($p=0.011$), hypertension ($p<0.0001$) and the use of beta-blockers ($p<0.0001$) were observed.

On-clopidogrel platelet reactivity and CCB treatment

► Figure 1 shows that users of amlodipine and Pgp-inhibiting CCBs exhibited higher on-clopidogrel platelet reactivity as compared to CCB non-users. On-clopidogrel platelet reactivity differed statistically significant between the three treatment groups ($p<0.0001$ for all platelet function assays). Pairwise comparisons showed that the mean ADP-induced on-clopidogrel maximal platelet aggregation was significantly higher in users of amlodipine as compared to CCB non-users ($41.9\% \pm 13.1$ vs. $36.7\% \pm 13.5$, for 5 μM , $p<0.0001$ and $60.3\% \pm 13.2$ vs. $54.5\% \pm 14.2$, for 20 μM ADP, $p<0.0001$, Fig. 1). After adjustment for the confounding variables, the use of amlodipine remained significantly associated with an increased on-clopidogrel platelet reactivity (mean difference: 4.4% [95% CI 1.6–7.2, $p=0.002$], for 5 μM ADP, and 5.0% [95% CI 2.1–7.9, $p=0.001$], for 20 μM). Likewise, the mean on-clopidogrel platelet reactivity when measured with VerifyNow P2Y12 assay was also significantly higher in users of amlodipine as compared to patients without CCB treatment (224.1 ± 73.4 vs. 191.1 ± 74.5 , $p<0.0001$, Fig. 1). The adjusted mean difference in VerifyNow P2Y12 PRU results between patients on amlodipine and CCB non-users was 26.9, 95% CI 11.9–36.2, $p<0.0001$.

Pairwise comparisons showed that the mean ADP-induced on-clopidogrel platelet reactivity for users of Pgp-inhibiting CCBs was also significantly higher as compared to patients without CCB treatment (Fig. 1). After adjustment for confounders, the use of Pgp-inhibiting CCBs remained significantly associated with an increased on-clopidogrel platelet reactivity (mean difference: 5.7% [95% CI 1.9–9.6, $p=0.003$], for 5 μM ADP, and 3.7% [95% CI 0.3–7.7, $p=0.035$] for 20 μM). However, platelet reactivity according to the VerifyNow P2Y12 assay did not differ between users of

Variable	No CCB, n=410	Pgp-inhibiting CCBs (diltiazem, nifedipine, verapamil, barnidipine), n=98	Amlodipine, n=124	P-value
Age (years)	62.5 ± 10.6	65.7 ± 10.0	64.7 ± 10.8	0.011
Men, n (%)	312 (76.1)	77 (78.6)	84 (67.7)	0.11
Body mass index (kg/m ²)	27.1 ± 3.9	27.0 ± 3.7	27.3 ± 3.6	0.81
Diabetes mellitus, n (%)	72 (17.6)	17 (17.3)	23 (18.5)	0.96
Current smokers, n (%)	52 (12.7)	6 (6.1)	9 (7.3)	0.07
Hypertension, n (%)	303 (73.9)	86 (87.8)	108 (87.1)	<0.0001
Hypercholesterolemia, n (%)	346 (84.4)	83 (84.7)	94 (76.4)	0.11
Family history of CAD, n (%)	251 (62.1)	59 (61.5)	75 (62.0)	0.99
Previous MI, n (%)	191 (47.3)	54 (56.8)	50 (41.3)	0.08
LVEF $<45\%$	66 (16.1)	13 (13.3)	18 (14.5)	0.33
Proton pump inhibitors, n (%)	94 (22.9)	29 (29.6)	34 (27.4)	0.30
CYP3A4-metabolised statins, n (%)	280 (68.3)	61 (62.2)	81 (65.3)	0.48
Beta-blockers, n (%)	349 (85.1)	62 (63.3)	114 (91.9)	<0.0001

Table 1: Baseline demographics and clinical characteristics of the study cohort. Data are expressed as mean value \pm SD or number of patients n (%); P-value: ANOVA for continuous variables and chi-square test for categorical variables between the three groups, CAD, coronary artery disease; MI, myocardial infarction; LVEF, left ventricular ejection fraction; CCB, calcium channel blocker; Pgp, P-glycoprotein.

Pgp-inhibiting CCBs and CCB non-users (204.5 ± 73.2 vs. 191.1 ± 74.5 , $p=0.11$, Fig. 1). No significant differences in platelet reactivity between users of Pgp-inhibiting CCBs and amlodipine were found in pairwise comparisons (Fig. 1).

Clopidogrel poor-response and CCB treatment

Based on LTA measurements, 97 patients (15.6% of the total cohort) were classified as clopidogrel poor-responders. The proportion of clopidogrel poor-responders was significantly higher in users of amlodipine compared to patients without CCB treatment (25.6% vs. 12.9%), resulting in an odds ratio of 2.3 (95% CI 1.4–3.9, $p=0.001$, ► Table 2). This association remained significant after the adjustment for confounders: adjusted OR (ORadj): 2.3, 95% CI 1.4–3.9, $p=0.001$. According to the VerifyNow P2Y12 assay, the use of amlodipine was associated with clopidogrel poor response (OR: 2.4, 95% CI 1.6–3.7, $p<0.0001$ and ORadj: 2.3, 95% CI 1.5–3.6, $p<0.0001$). In the group of patients on Pgp-inhibiting CCBs, 14.6% was classified as clopidogrel poor-responder. No association between the risk of clopidogrel poor-response and the use of Pgp-inhibiting CCBs was found (OR: 1.2, 95% CI 0.6–2.2, $p=0.66$ and ORadj 0.9, 95% CI 0.4–2.2, $p=0.92$, Table 2). In concordance with results from the LTA, no association between concomitant use of Pgp-inhibiting CCBs and clopidogrel poor-response as measured with the VerifyNow P2Y12 assay was found (OR: 1.3, 95% CI 0.8–2.1, $p=0.33$ and ORadj: 1.5, 95% CI 0.8–2.8, $p=0.19$, Table 2). Sub-analysis within the group of Pgp-inhibiting CCBs showed that the dihydropyridins nifedipine and barnidipine ($n=40$) did not have any influence on clopidogrel poor-responder status (LTA: OR 1.4, 95% CI 0.6–3.4, $p=0.41$ and ORadj 1.4, 95% CI 0.6–3.4, $p=0.49$ and VerifyNow: OR 1.0, 95% CI 0.5–2.1, $p=0.97$ and ORadj 0.9, 95% CI 0.4–2.1, $p=0.96$). The use of diltiazem and verapamil ($n=58$) also did not have any influence on clopidogrel poor-response (LTA: OR 1.0, 95% CI 0.4–2.3, $p=0.94$ and ORadj 1.0, 95% CI 0.4–2.3, $p=0.91$ and VerifyNow: OR 1.5, 95% CI 0.8–2.7, $p=0.17$ and ORadj 1.5, 95% CI 0.8–2.9, $p=0.24$).

Table 2: Odds ratios (OR) for poor-responder status according to CCB treatment. ORs with 95% confidence intervals for poor-responder status according to CCB treatment. Poor-responder: clopidogrel pre-treated subject with more than 70% aggregation to 20 μ M ADP (LTA) or more than 235 P2Y12 Reaction Units (PRU, VerifyNow). CCBs, calcium channel blockers; # Multivariate analysis: adjusted for gender, age, body mass index, diabetes mellitus, previous myocardial infarction, LVEF <45%, hypertension, current smoking, duration of clopidogrel administration before the coronary stent

CCB	Poor-responder	Crude OR [95% CI]	P-value	Adjusted OR [95% CI]	P-value
Amlodipine	LTA – ADP	2.3 [1.4–3.9]	0.001	2.3 [1.4–3.9]	0.001
	VerifyNow – PRU	2.4 [1.6–3.7]	<0.0001	2.3 [1.5–3.6]	<0.0001
Pgp-inhibiting CCBs	LTA – ADP	1.2 [0.6–2.2]	0.66	0.9 [0.4–2.2]	0.92
	VerifyNow – PRU	1.3 [0.8–2.1]	0.33	1.5 [0.8–2.8]	0.19

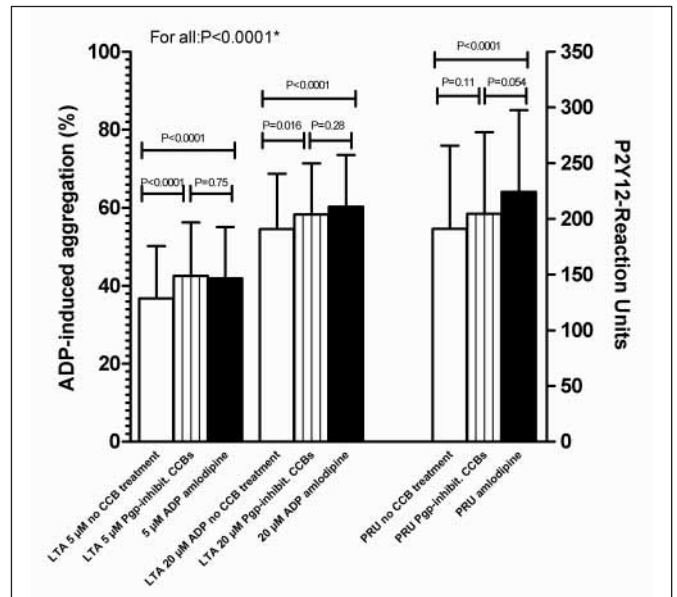


Figure 1: On-clopidogrel platelet reactivity according to the use of calcium channel blockers.

Discussion

In the present study we assessed the influence of the concomitant use of different CCBs on on-clopidogrel platelet reactivity in a large cohort of patients on dual antiplatelet therapy undergoing elective PCI. Co-administration of amlodipine, which does not inhibit Pgp, was associated with increased on-clopidogrel platelet reactivity. Furthermore, the use of amlodipine was associated with an 2.3-fold increased risk of clopidogrel poor-response using the predefined criteria for poor-response as more than 70% platelet aggregation to 20 μ M ADP (LTA) or a VerifyNow P2Y12 PRU-value of more than 235. In other studies, this parameter is associated with adverse cardiovascular events, including stent thrombosis (24, 25). The use of the Pgp-inhibiting CCBs diltiazem, verapa-

implantation (in days) and the use of proton pump inhibitors. Figure 1: On-clopidogrel platelet reactivity according to the use of calcium channel blockers. Platelet aggregation (as measured with LTA after 5 and 20 μ M ADP and VerifyNow P2Y12 assay) in patients with no CCB treatment (open bars), treatment with Pgp-inhibiting CCBs (striped bars) and with amlodipine (non-Pgp-inhibiting CCB) (solid bars). CCB, calcium channel blocker; ADP, adenosine diphosphate. *ANOVA with LSD post-hoc tests.

mil, barnidipine and nifedipine was found to increase on-clopidogrel platelet reactivity as measured with LTA. However, no influence was observed when platelet reactivity was measured with the VerifyNow P2Y12 assay and importantly, this subclass of CCBs was not associated with an increased risk of clopidogrel poor-response.

The inhibitory effect of CCBs on the platelet response to clopidogrel is thought to be caused at the level of CYP3A4 (18). Clopidogrel is a prodrug, which requires hepatic biotransformation by CYP3A4 to generate the active metabolite (3). As all CCBs are substrates and inhibitors of CYP3A4 (20), concomitant use could inhibit clopidogrel's metabolism. The intestinal absorption of clopidogrel is limited by P-glycoprotein by increasing the intestinal efflux (2). The CCBs verapamil, diltiazem, nifedipine and barnidipine are potent inhibitors of Pgp and have been shown to increase the responsiveness to several drugs e.g. digoxin and anticancer agents, by this mechanism (17, 26–29). Inhibition of Pgp by the concomitant use of Pgp-inhibiting CCBs may lead to a decreased intestinal efflux of clopidogrel, thereby increasing clopidogrel plasma concentrations and counteracting the effect of CCB-induced CYP3A4 inhibition. Therefore, concomitant use of Pgp-inhibiting CCBs might have less clinical relevance than co-administration of amlodipine. However, the clinical use of diltiazem and verapamil is not completely comparable with the use of amlodipine. Amlodipine is solely used in the treatment of hypertension and coronary artery disease while diltiazem and verapamil are also used for rate control in atrial fibrillation. However, sub-analysis within the group of Pgp-inhibiting CCBs showed that nifedipine and barnidipine, drugs that have the same clinical use as amlodipine, also have no influence on clopidogrel poor-response. In the study of Siller-Matula et al., co-administration of CCBs was found to be associated with a diminished pharmacodynamic response to clopidogrel and with an increased risk of adverse cardiovascular events (18). The authors made no distinction between the different CCBs but the majority of their study population used amlodipine. These results are consistent with our observation that the use of amlodipine is associated with clopidogrel poor-response. In our study, amlodipine was the only representative of the CCB subclass with no inhibiting effect on Pgp. Other non-Pgp-inhibiting CCBs like nimodipine, nisoldipine and isradipine, were not studied. There are some limitations of this study. First, in this observational study, we cannot completely exclude possible bias by various risk factors and patient characteristics although the multivariable adjustment models confirmed the primary analyses. Furthermore, we did not investigate the influence of CCBs on plasma concentrations of clopidogrel's active metabolite nor on clinical outcome. An additional limitation is that we did not adjust for the carriage of genetic variants of e.g. CYP2C9, CYP2C19 and Pgp, which are found to play a role in the antiplatelet properties of clopidogrel.

In conclusion, concomitant use of Pgp-inhibiting CCBs and amlodipine increases on-clopidogrel platelet reactivity. However, only amlodipine was associated with a higher risk of clopidogrel poor-response. These findings may have important implications with regard to which type of CCB is preferred in clopidogrel-treated patients.

References

- Mehta SR, Yusuf S, Peters RJ, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* 2001; 358: 527–533.
- Taubert D, von Beckerath N, Grimberg G, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006; 80: 486–501.
- Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360: 354–362.
- Pereillo JM, Maftouh M, Andrieu A, et al. Structure and stereochemistry of the active metabolite of clopidogrel. *Drug Metab Dispos* 2002; 30: 1288–1295.
- Savi P, Pereillo JM, Uzabiaga MF, et al. Identification and biological activity of the active metabolite of clopidogrel. *Thromb Haemost* 2000; 84: 891–896.
- Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005; 45: 246–251.
- Hulot JS, Bura A, Villard E, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006; 108: 2244–2247.
- Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008; 51: 1925–1934.
- Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363–375.
- Sibbing D, Stegherr J, Latz W, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009; 30: 916–922.
- Collet JP, Hulot JS, Pena A, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009; 373: 309–317.
- Giusti B, Gori AM, Marcucci R, et al. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol* 2009; 103: 806–811.
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. *Arterioscler Thromb Vasc Biol* 2006; 26: 1895–1900.
- Suh JW, Koo BK, Zhang SY, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *Cmaj* 2006; 174: 1715–1722.
- Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007; 5: 2429–2436.
- Harmsze A, van Werkum JW, Bouman HJ, et al. Besides CYP2C19*2, the variant allele CYP2C9*3 is associated with higher on-clopidogrel platelet reactivity in patients on dual antiplatelet therapy undergoing elective coronary stent implantation. *Pharmacogenet Genomics* 2010; 20: 18–25.
- Katoh M, Nakajima M, Yamazaki H, et al. Inhibitory potencies of 1,4-dihydropyridine calcium antagonists to P-glycoprotein-mediated transport: comparison with the effects on CYP3A4. *Pharm Res* 2000; 17: 1189–1197.
- Siller-Matula JM, Lang I, Christ G, et al. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. *J Am Coll Cardiol* 2008; 52: 1557–1563.
- Gremmel T, Steiner S, Seidinger D, et al. Calcium-channel blockers decrease clopidogrel-mediated platelet inhibition. *Heart* 2010; 96: 186–189.
- Zhou SF, Xue CC, Yu XQ, et al. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther Drug Monit* 2007; 29: 687–710.
- von Beckerath N, Pogatsa-Murray G, Wiecek A, et al. Correlation of a new point-of-care test with conventional optical aggregometry for the assessment of clopidogrel responsiveness. *Thromb Haemost* 2006; 95: 910–911.
- van Werkum JW, van der Stelt CA, Seesing TH, et al. A head-to-head comparison between the VerifyNow P2Y12 assay and light transmittance aggregometry for monitoring the individual platelet response to clopidogrel in patients undergoing elective percutaneous coronary intervention. *J Thromb Haemost* 2006; 4: 2516–2518.
- van Werkum JW, Harmsze AM, Elsenberg EH, et al. The use of the VerifyNow system to monitor antiplatelet therapy: a review of the current evidence. *Platelets* 2008; 19: 479–488.

24. Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006; 27: 2420–2425.
25. Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008; 29: 992–1000.
26. Wachter VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* 1995; 13: 129–134.
27. Mahgoub AA, El-Medany AH, Abdulatif AS. A comparison between the effects of diltiazem and isosorbide dinitrate on digoxin pharmacodynamics and kinetics in the treatment of patients with chronic ischemic heart failure. *Saudi Med J* 2002; 23: 725–731.
28. Kirch W, Hutt HJ, Dylewicz P, et al. Dose-dependence of the nifedipine-digoxin interaction? *Clin Pharmacol Ther* 1986; 39: 35–39.
29. Rodin SM, Johnson BF, Wilson J, et al. Comparative effects of verapamil and isradipine on steady-state digoxin kinetics. *Clin Pharmacol Ther* 1988; 43: 668–672.