Thrombosis Research 129 (2012) e36–e40

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Lack of association between serum paraoxonase-1 activity and residual platelet aggregation during dual anti-platelet therapy

Tsukasa Ohmori [a,*,1], Yuichiro Yano [b,1], Asuka Sakata [a,b], Tomokazu Ikemoto [b], Masahisa Shimpo [b], Seiji Madoiwa [a], Takaaki Katsuki [b], Jun Mimuro [a], Kazuyuki Shimada [b], Kazuomi Kario [b,2], Yoichi Sakata [a,*,2]

[a] Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University School of Medicine, 3311–1 Yakushiji, Shimotsuke, Tochigi 329–0498, Japan
[b] Division of Cardiovascular Medicine, Department of Medicine, Jichi Medical University School of Medicine, 3311–1 Yakushiji, Shimotsuke, Tochigi 329–0498, Japan

ARTICLE INFO

Article history:
Received 3 August 2011
Received in revised form 18 October 2011
Accepted 30 October 2011
Available online 25 November 2011

Keywords:
antiplatelet agent
platelet pharmacology
coronary syndrome

ABSTRACT

High residual platelet aggregability during thienopyridine treatment occurs because of low levels of the active drug metabolite, and is associated with an increased rate of major adverse cardiovascular events. Recent findings suggest that paraoxonase-1 (PON1) is a major determinant for clopidogrel efficacy. The aim of this study was to assess the impact of serum PON1 activity on platelet aggregability in thienopyridine-treated patients. In 72 patients receiving treatment with aspirin and ticlopidine after acute coronary syndrome, various laboratory data including the formation of platelet aggregations induced by agonists were compared with serum PON1 activities, measured as paraoxonase and homocysteine thiolactone hydrolase (HTLase). Serum paraoxonase activity was significantly associated with HTLase activity ($R=0.4487$, $P<0.0001$). These PON1 activities were not correlated with any parameters for platelet aggregation, hypertension, sleep apnea, and diabetes mellitus. In contrast, serum PON1 activities seemed to be involved in cardiac function, with brain natriuretic peptide and ejection fraction being significantly correlated with serum HTLase activity ($R=-0.2767$, $P=0.0214$) and paraoxonase activity ($R=0.2558$, $P=0.0339$), respectively. Paraoxonase activity also demonstrated a significant association with increased levels of ankle-brachial index ($R=0.267$, $P=0.0255$). Serum PON1 activities did not influence platelet aggregability during treatment with thienopyridine. However, they might modulate cardiac function after acute coronary syndrome and progression of atherosclerosis.

Introduction

The concept of antiplatelet resistance, particularly poor responsiveness to thienopyridine, has received increasing attention in recent years because of its reported involvement in cardiovascular events after percutaneous coronary artery intervention (PCI) [1-3]. Thienopyridines such as clopidogrel and ticlopidine are rapidly absorbed prodrugs, and must therefore be converted to an active metabolite to exert their inhibitory actions at the target $P2Y_{12}$ ADP nucleotide receptor on platelets. This conversion is *via* a two-step process involving the hepatic cytochrome P450 (CYP) enzyme pathway [4]. Resistance to clopidogrel was thought to result mainly from decreased CYP function leading to reduced active metabolite production [4]. Indeed, individuals carrying the loss-of-function polymorphism of the *CYP2C19* allele had significantly lower levels of the active metabolite of clopidogrel, and a higher rate of major adverse cardiovascular events [5,6]. Drug interaction with the CYP2C19 inhibitor, omeprazole, might also reduce the production of active metabolites [7,8].

Very recently, it was reported that paraoxonase-1 (PON1) is a major and essential factor in the production of active metabolites from clopidogrel [9]. PON1 hydrolyses 2-oxoclopidogrel (an oxidative metabolite of clopidogrel) to form the final active metabolite, a thiol derivative of clopidogrel (Supplemental Fig. 1) [9]. PON1 is a high-density lipoprotein-associated enzyme that prevents oxidative modification of low-density lipoprotein [10]. The *PON1* genotype (Q192 allele) has significant dose-dependent associations with decreased levels of serum PON1 activity and with increased levels of oxidative stress [11]. PON1 has multiple enzyme activities including paraoxonase, arylesterase, and thiolactonase (Supplemental Fig. 1). Although the full range of endogenous substrates hydrolysed by PON1 remains to be elucidated, PON1 has been shown to produce homocysteine from homocysteine thiolactone *via* its homocysteine thiolactone hydrolase (HTLase) activity [12].

Abbreviations: PON1, paraoxonase-1; HTLase, homocysteine thiolactone hydrolase; PCI, percutaneous coronary artery intervention; CYP, cytochrome P450; BNP, brain natriuretic peptide.

* Corresponding authors at: Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University School of Medicine, 3111–1 Yakushiji, Shimotsuke, Tochigi 329–0498, Japan. Tel.: +81 285 58 7397; fax: +81 285 44 7817.
E-mail addresses: tohmori@jichi.ac.jp (T. Ohmori), yoisaka@jichi.ac.jp (Y. Sakata).

[1] TO and YY equally contributed in this study.
[2] KK and YS are co-senior authors due to equal contribution.

0049-3848/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2011.10.033

We have previously investigated the mechanisms and clinical backgrounds that determine residual platelet aggregability, and attempted to ascertain whether platelet aggregability is involved in systemic thrombogenicity during dual antiplatelet therapy [13]. Using this previous population, we have retrospectively measured actual serum PON1 activities, measured as paraoxonase and HTLase, in 72 patients treated with ticlopidine and aspirin, and assessed the correlation between PON1 and platelet aggregability.

Methods

Patients

The institutional review board approved all study protocols, and informed consent was obtained from all participants. The design and protocol of this study has been described previously [13]. Briefly, we enrolled consecutive hospitalized patients from July 2006 to April 2007 who were treated by PCI because of symptomatic coronary artery disease. After normalization of cardiac enzymes, patients underwent blood sampling, ankle-brachial index monitoring and cardiorespiratory monitoring.

Blood collection and platelet aggregation

Platelet aggregation was assessed as described previously [14]. A fasting venous sample was carefully collected, and platelet-rich plasma was obtained by centrifugation. The aggregation response was measured based on the light scattering intensities obtained with a PA-200 platelet aggregation analyzer (Kowa Co. Ltd., Tokyo, Japan). This device is particularly sensitive for detecting and classifying the size of platelet aggregates (small, medium, and large) [14]. Platelet aggregation was stimulated with collagen (Hormon-Chemie, Munich, Germany), ADP (MC Medical Co., Tokyo, Japan) and thrombin receptor-activating peptide (TRAP; Invitrogen Co., Carlsbad, CA), a specific agonist for protease activating receptor-1. Blood samples (serum and plasma) were stored at −80 °C until analysis.

Laboratory testing

Plasma levels of plasminogen activator inhibitor-1 antigen, D-dimer, E-selectin and soluble fibrin were assayed using an automated latex agglutination assay (LPIA-S500; Mitsubishi Chemical Medience Co., Tokyo, Japan) based on conjugated monoclonal antibodies. The concentrations of brain natriuretic peptide (BNP) (ShionoRIA BNP kit; Shionogi USA, Inc. Florham Park, NJ) were measured by SRL Inc. (Tokyo, Japan).

Measurement of serum PON1 activities

We quantified paraoxonase and HTLase activities as a measure of serum PON1 activity (Supplemental Fig. 1). Serum paraoxonase activity was measured by using paraoxon as a substrate (Fully Automated Paraoxonase Activity Measurement Kit, Rel Assay Diagnostics, Gaziantep, Turkey). HTLase activity was measured by a hydrolysis of γ-thiobutyrolactone (Alfresa Auto HTLase, Alfresa Pharma Corp., Osaka, Japan). HTLase hydrolyzes the lactone ring of the substrate γ-thiobutyrolactone, producing free thiols that are detected using Ellman's reagent (DTNB; 5,5'-dithiobis (2-nitrobenzoic acid)). Assay reproducibility was high (coefficient of variation was less than 6%).

Statistical analysis

Statistical analyses were performed using Prism v5 (GraphPad software, Inc, La Jolla, CA). The associations between the individual parameters were calculated using Spearman's correlation method. All reported *P* values are two-sided; a *P* value of less than 0.05 was considered to indicate statistical significance.

Results

Patients

Of the 85 patients from our previous study, we selected 72 patients taking 100 mg / day of aspirin and 200 mg / day of ticlopidine after acute coronary syndrome. Base line characteristics of the study population are summarized in Table 1.

Lack of correlation of serum PON1 activities with platelet aggregation

We initially examined serum PON1 activities (measured by paraoxonase and HTLase activity). As show in Fig. 1, serum HTLase activity, but not paraoxonase activity, appeared to be normally distributed across the study population (HTLase: 130.3 ± 36.7 U/L; paraoxonase: 62.65 ± 25.27 U/L). These PON1 activities were significantly correlated ($R = 0.4487$, $P < 0.0001$). To examine whether serum PON1 activities determine platelet aggregability during dual antiplatelet therapy, serum PON1 activities were compared with several parameters of platelet aggregation. However, none of these parameters was significantly associated with PON1 activities (Fig. 2 and Table 2).

Correlation between serum PON-1 activities and cardiac function

We next compared serum PON1 activities with parameters for hypertension, sleep apnea, diabetes mellitus, hyperlipidemia, blood coagulation, arteriosclerosis, and cardiac dysfunction. Using linear regression analysis, we determined that only HDL cholesterol and BNP were correlated with HTLase activity (Table 3). Paraoxonase activity was associated with triglyceride, D-dimer, ankle-brachial index, and ejection fraction (Table 3). The medication including use of diuretics, angiotensin II receptor blocker, angiotensin converting enzyme inhibitor, beta blocker, calcium channel blocker, or statin did not demonstrate a significant association with serum PON1 activities (Supplemental Table 1). These data suggest that decreased levels of PON1 activity might lead to the acceleration of atherosclerosis and cardiac dysfunction after acute coronary syndrome.

Table 1
Characteristics of the study population.

Variables	Total subjects (n = 72)
Age, years	62.15 ± 11.62
Men, n (%)	57 (80)
BMI, kg/m^2	25.11 ± 3.514
Systolic blood pressure (mmHg)	125.3 ± 21.08
Diastolic blood pressure (mmHg)	76.6 ± 11.28
Pulse rate (/min)	72.39 ± 14.59
Blood sugar (mg/dl)	118.3 ± 50.43
HbA1c (%)	6.76 ± 1.891
Triglyceride (mg/dl)	130.2 ± 53.01
Total cholesterol (mg/dl)	167.7 ± 36.77
LDL cholesterol (mg/dl)	100.4 ± 30.35
HDL cholesterol (mg/dl)	41.3 ± 12.72
CPK max (U/L)	2,194 ± 2,211
BNP (pg/ml)	151.4 ± 183.6
Concomitant medications	
Antiplatelet agents, n (%)	
Aspirin + Ticlopidine	72 (100)
Antihypertensive medication, n (%)	66 (91.7)
Statin, n (%)	55 (76.4)
NSAIDs, n (%)	0 (0)

Data for continuous variables are expressed as the mean ± SD. BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BNP, brain natriuretic peptide; NSAID, non-steroidal anti-inflammatory drug.

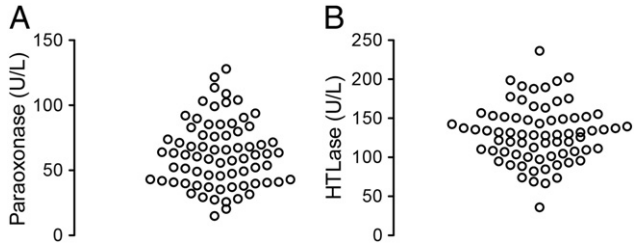

Fig. 1. Serum paraoxonase and HTLase activities in the study population.

Discussion

Inhibition of the $P2Y_{12}$ nucleotide receptor, an ADP receptor on platelets, is currently the gold-standard therapy for the prevention of ischemic events in patients undergoing PCI [15,16]. Although the second-generation thienopyridine, clopidogrel, is recommended by a number of current clinical guidelines, the inter-individual variability of its efficacy is a major drawback in its clinical use [17]. Better understanding of this variability in the efficacy of clopidogrel and other thienopyridines is vital at a time when the number of PCIs is increasingly rapidly. The loss-of-function polymorphism of the CYP2C19 allele has attracted attention as a potential factor in clopidogrel efficacy [4-6], while an elegant recent study suggested that PON1 is a major determinant in the production of the final active metabolite of clopidogrel [9]. In this study, we measured serum two PON1 activities in acute coronary syndrome and compared them with platelet aggregability in patients receiving dual antiplatelet therapy. We could identify no correlation between PON1 activities and any parameter for platelet aggregation in our population.

Several explanations may exist for the discrepancy between our result and the previous report. First, genetic divergence between Caucasian and Japanese patients might affect the result. The Japanese population is reported to express predominantly the 192R allele of *PON1* (192QQ: 18.2%; 192QR: 40.9%; 192RR: 40.9%) [18], whereas the Caucasian population in a large cohort study tended to express the 192Q variant (192QQ: 46.3%; 192QR: 43.9%; 192RR: 9.8%) [11]. The Q allele of *PON1* genotype was significantly and dose-dependently associated with decreased serum PON1 activity, whereby 192QQ, 192QR and 192RR had comparatively low, intermediate and high PON1 activity, respectively [11]. In contrast, the frequency of polymorphism for *CYP2C19*, a key enzyme in clopidogrel oxidation, varies among races, with loss-of-function polymorphisms reportedly being more common in Asian patients [19,20]. However, even in a genetically homogenous population, the *CYP2C19* allele was reported to account for only 12% of the variability in clopidogrel efficacy, whereas the *PON1*

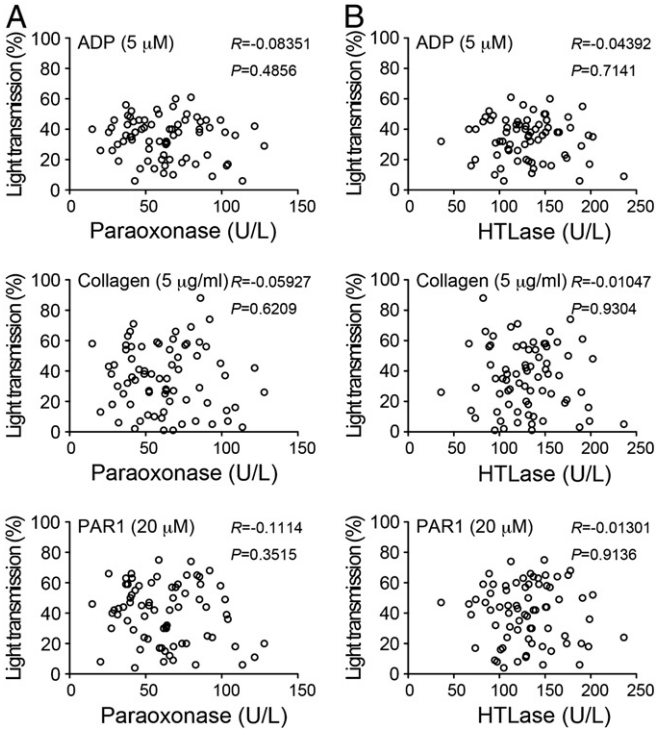

Fig. 2. Association between PON1 activities and platelet aggregation. Platelet aggregation induced by 5 μM ADP, or 5 μg / ml of collagen, or 20 μM TRAP was assessed by aggregometry, and was expressed as light transmission (%). Serum paraoxonase activities (U / L) (A) and HTLase activities (U / L) (B) were compared with platelet aggregation using Spearman's rank correlation coefficient.

Table 2
Correlation between serum paraoxonase activities and platelet aggregation.

	Paraoxonase		HTLase	
	R	*P* value	*R*	*P* value
ADP 2 μM-LT	−0.1252	0.2945	−0.03844	0.7486
ADP 2 μM-Small	−0.2083	0.0791	−0.07811	0.5143
ADP 2 μM- Med	−0.1335	0.2636	−0.01776	0.8823
ADP 2 μM-Large	−0.03798	0.7514	0.085	0.4777
ADP 5 μM-LT	−0.08351	0.4856	−0.04392	0.7141
ADP 5 μM-Small	−0.2212	0.0619	−0.09755	0.415
ADP 5 μM- Med	−0.2317	0.0501	−0.1055	0.3776
ADP 5 μM-Large	−0.1406	0.2389	−0.06589	0.5824
Coll 1 μg/ml-LT	−0.1072	0.37	0.02695	0.8222
Coll 1 μg/ml-Small	−0.1524	0.2012	0.06594	0.5821
Coll 1 μg/ml- Med	−0.1174	0.3262	0.04772	0.6906
Coll 1 μg/ml-Large	−0.00214	0.9857	0.04327	0.7182
Coll 5 μg/ml-LT	−0.05927	0.6209	−0.01047	0.9304
Coll 5 μg/ml-Small	−0.1489	0.212	−0.1001	0.4029
Coll 5 μg/ml- Med	−0.1269	0.2881	−0.03555	0.7669
Coll 5 μg/ml-Large	−0.1113	0.352	0.007927	0.9473
TRAP 20 μM-LT	−0.1114	0.3515	0.01301	0.9136
TRAP 20 μM -Small	−0.1585	0.1835	−0.1742	0.1434
TRAP 20 μM - Med	−0.09187	0.4427	−0.04854	0.6855
TRAP 20 μM -Large	−0.05235	0.6623	0.03127	0.7943

LT, light transmission; Small, small aggregates; Med, medium aggregates; Large, Large aggregates; Coll, collagen; TRAP, thrombin receptor-activating peptide (SFLLRN). *$P<0.05$.

Table 3
Correlation between paraoxonase activities and other laboratory data.

	Paraoxonase		HTLase	
	R	*P* value	*R*	*P* value
SBP	−0.0953	0.443	−0.118	0.3417
DBP	−0.121	0.3294	−0.00797	0.949
HR	−0.1096	0.3774	0.02895	0.8161
AHI	0.06843	0.5735	0.05563	0.6474
Blood suger	−0.06266	0.609	0.06714	0.5836
HbAlc	−0.08087	0.5121	−0.09806	0.4263
Triglyceride	0.2958	0.0129*	0.00273	0.9821
Total cholesterol	0.01199	0.9215	0.1166	0.3365
LDL cholesterol	−0.0158	0.8975	0.05602	0.6475
HDL cholesterol	−0.01299	0.915	0.2646	0.0269*
PAI-1	−0.08214	0.4928	0.01523	0.899
E-selectin	−0.0007075	0.9953	−0.1618	0.1746
Soluble Fibrin	−0.03224	0.788	−0.08483	0.4787
D-dimer	−0.2348	0.0471*	−0.2229	0.0598
max CPK	−0.1691	0.1616	−0.02521	0.8359
BNP	−0.1306	0.2849	−0.2767	0.0214*
Pulse wave velocity	−0.1665	0.1682	−0.04456	0.7141
ABI	0.267	0.0255*	−0.01957	0.8722
Ejection fraction	0.2558	0.0339*	0.1632	0.1803

SBP, systolic blood pressure; DBP, diastolic blood pressure; AHI, apnea-hypopnea index; LDL, low-density lipoprotein; HDL, high-density lipopurotein; PAI-1, plasminogen activator inhibitor-1; BNP, brain natriuretic peptide; ABI, ankle-brachial index. *$P<0.05$.

Q192R polymorphism was estimated to be responsible for 72.5% of the variability in ADP-stimulated platelet aggregation after clopidogrel administration [9]. It is therefore important that we clarify which polymorphism combinations (of *PON1* and *CYP2C19*) are the most relevant in the metabolism of thienopyridines in our population.

The first-generation thienopyridine, ticlopidine, was used instead of clopidogrel in our study because ticlopidine was the only approved drug for acute coronary syndrome in Japan during our study period. We acknowledge the possibility that the rate-limiting enzyme for ticlopidine metabolism to its active metabolite may differ from that of clopidogrel. All thienopyridines including ticlopidine, clopidogrel, and prasugrel are prodrugs that need to be converted into active metabolite through the formation of thiolactone metabolites (2-oxo-ticlopidine, 2-oxo-cloipdogrel, and prasugrel thiolactone, respectively (see Supplemental Fig. 1)) [4]. The free active thiol of these active metabolites forms disulfide bonds with, and therefore binds irreversibly to, cysteine residues Cys17 and Cys270 of $P2Y_{12}$ [21]. It is of great importance, therefore, to understand whether thiolactone metabolites of all thienopyridines are hydrolyzed mainly by PON1, or are instead oxidized by CYP.

We found correlations between PON1 activities and cardiac function in our study population. PON1 has a protective effect against oxidation of lipoproteins, and a *PON1* polymorphism (the 192Q allele) that produces decreased levels of PON1 activity was associated with systemic oxidative stress and higher rates of major cardiovascular events [11]. It is possible that decreased levels of PON1 activities enhance the progression of atherosclerosis in the coronary artery, resulting in decreased cardiac function after acute coronary syndromes. Indeed, reduced paraoxonase activity was significantly associated with a decreased ankle-brachial index in our study. Further studies are needed to assess the possible mechanisms and biological effect of PON1, particularly the severity of its effects on cardiac function after coronary artery disease.

Some limitations in this study merit discussion. First, we could assess platelet function testing in the patients treated with ticlopidine, but not clopidogrel. We cannot exclude the possibility that results may differ with other thienopyridines, as described above. In addition, we assessed only the correlation between serum PON1 activities and platelet response to ticlopidine, and we did not assess gene polymorphisms. Although it is accepted that serum PON1 activities are determined by *PON1* polymorphism, more data regarding genetic variation in *CYP* and *PON1* may have extended our findings relating to the mechanism(s) of the platelet response during dual antiplatelet therapy. Finally, the analysis reported here is *post hoc* analysis of a previously reported population and the number of participants is limited. We previously estimated that at least 62–85 participants would be required for the study ($\alpha = 0.05$, $\beta = 0.20$, and expected correlation coefficient, $R = 0.30$–0.35) [13]. Weak association due to β-error may affect the strength of any conclusions based on these data.

Conclusions

The current study has demonstrated that serum PON1 activities did not influence platelet aggregation in patients receiving thienopyridine treatment, but was involved in cardiac function. Our data suggest the need for a re-evaluation of the importance of PON1 (and/or CYP) in the production of active metabolites from thienopyridines. We may also need to consider how expression of the rate-limiting enzymes for thienopyridine metabolism differs between individual drugs and racial populations. During the preparation of this article, it was reported that no association exists between *PON1* genotype and platelet response to clopidogrel and stent thrombosis in a *post hoc* analysis of prospective studies [22]. Further large-scale prospective studies are required to determine which enzyme (PON1 or CYP) is critical for the production of active metabolites from thienopyridines, and therefore for cardiovascular events during thienopyridine administration.

Supplementary materials related to this article can be found online at doi:10.1016/j.thromres.2011.10.033.

Conflict of interests statement

T.O. has received financial support from Daiichi Sankyo. The other authors declare that they have no competing interest.

Acknowledgements

We would like to acknowledge Dr. Y. Yatomi (The University of Tokyo) for very helpful discussions. The authors are grateful for the hard work of the Coronary Care Unit staff in patient recruitment and management. We also thank N. Matsumoto and M. Ito for their excellent technical assistance. This study was supported by grants from the Support Program for Strategic Research Infrastructure from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

[1] Alexopoulos D. Clopidogrel pretreatment in PCI: absolute requirement or obsolete myth? Int J Cardiol 2011;146:305–10.
[2] Farre AJ, Tamargo J, Mateos-Caceres PJ, Azcona L, Macaya C. Old and new molecular mechanisms associated with platelet resistance to antithrombotics. Pharm Res 2010;27:2365–73.
[3] Maree AO, Fitzgerald DJ. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation 2007;115:2196–207.
[4] Cattaneo M. The platelet P2Y receptor for adenosine diphosphate: congenital and drug-induced defects. Blood 2011;117:2102–12.
[5] Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med 2009;360:354–62.
[6] Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 2009;360:363–75.
[7] Sibbing D, Morath T, Stegherr J, Braun S, Vogt W, Hadamitzky M, et al. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. Thromb Haemost 2009;101:714–9.
[8] O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. Lancet 2009;374:989–97.
[9] Bouman HJ, Schomig E, van Werkum JW, Velder J, Hackeng CM, Hirschhauser C, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. Nat Med 2011;17:110–6.
[10] Goswami B, Tayal D, Gupta N, Mallika V. Paraoxonase: a multifaceted biomolecule. Clin Chim Acta 2009;410:1–12.
[11] Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA 2008;299:1265–76.
[12] Ferretti G, Bacchetti T, Marotti E, Curatola G. Effect of homocysteinylation on human high-density lipoproteins: a correlation with paraoxonase activity. Metabolism 2003;52:146–51.
[13] Yano Y, Ohmori T, Hoshide S, Madoiwa S, Yamamoto K, Katsuki T, et al. Determinants of thrombin generation, fibrinolytic activity, and endothelial dysfunction in patients on dual antiplatelet therapy: involvement of factors other than platelet aggregability in Virchow's triad. Eur Heart J 2008;29:1729–38.
[14] Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J, et al. Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemost 2006;4:1271–8.
[15] Becker RC, Meade TW, Berger PB, Ezekowitz M, O'Connor CM, Vorchheimer DA, et al. The primary and secondary prevention of coronary artery disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;133:776S–814S.
[16] Wright RS, Anderson JL, Adams CD, Bridges CR, Casey Jr DE, Ettinger SM, et al. 2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction (Updating the 2007 Guideline) A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American College of Emergency Physicians, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol 2011;57:1920–59.
[17] Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol 2007;50:1822–34.
[18] Togawa T, Mukai Y, Ohata K, Suzuki T, Tanabe S. Measurement of homocysteine thiolactone hydrolase activity using high-performance liquid chromatography with fluorescence detection and polymorphisms of paraoxonase in normal human serum. J Chromatogr B Analyt Technol Biomed Life Sci 2005;819:67–72.

[19] Kim KA, Song WK, Kim KR, Park JY. Assessment of CYP2C19 genetic polymorphisms in a Korean population using a simultaneous multiplex pyrosequencing method to simultaneously detect the CYP2C19*2, CYP2C19*3, and CYP2C19*17 alleles. J Clin Pharm Ther 2010;35:697–703.
[20] Jinnai T, Horiuchi H, Makiyama T, Tazaki J, Tada T, Akao M, et al. Impact of CYP2C19 polymorphisms on the antiplatelet effect of clopidogrel in an actual clinical setting in Japan. Circ J 2009;73:1498–503.
[21] Ding Z, Kim S, Dorsam RT, Jin J, Kunapuli SP. Inactivation of the human P2Y12 receptor by thiol reagents requires interaction with both extracellular cysteine residues, Cys17 and Cys270. Blood 2003;101:3908–14.
[22] Sibbing D, Koch W, Massberg S, Byrne RA, Mehilli J, Schulz S, et al. No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. Eur Heart J 2011;32:1605–13.