THE OVER-ESTIMATED ROLE OF PON1 AND ITS VARIANTS IN CLOPIDOGREL BIOACTIVATION AND CLINICAL OUTCOMES: AN UPDATE

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REVIEW

HIGHLIGHTS

- PON1 catalyzes the formation of a minor endothiol metabolite of clopidogrel – "endo", not of its major active metabolite "H4". Thus, PON1 plays a less important role in clopidogrel bioactivation than CYP2C19 and other P450s involved.
- The definite effects of PON1 and its variants on clopidogrel bioactivation and platelet responses could be deciphered after further exclusion of the relative contribution of CYP2C19 in the subjects treated with clopidogrel.
- If PON1 activity levels do not correlate well with plasma H4 concentrations, exploration of the effects of PON1 and its variants on clopidogrel bioactivation and clinical outcomes would lose their theoretic support.
- The effects of PON1 and its genetic variants on clopidogrel clinical outcomes have been over-estimated in most clinical research studies.

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ABSTRACT

Clopidogrel is frequently used in the clinical settings. The two-step metabolic pathways are required for clopidogrel bioactivation in the liver before it exerts its antiplatelet effects. In addition to CYP2C19-mediated metabolism, PON1-catalyzed hydrolysis was considered as the major determinant of clopidogrel bioactivation in an earlier study. However, most of the subsequent studies argued about such a finding. Logically, if PON1 activity is not correlated with clopidogrel bioactivation, the effects of PON1 and its variants on clopidogrel platelet responses would be less important; and if the role of CYP2C19 in clopidogrel bioactivation is not ruled out, the effects of PON1 and its genetic polymorphisms on clopidogrel platelet responses could not be true. In this review, we systematically summarized current status about the potential interactions of PON1 (and its variants) with clopidogrel in vitro and/or in vivo, and found that the role of PON1 in clopidogrel bioactivation has been over-estimated.

KEY WORDS

Clopidogrel; CYP2C19; platelet aggregation; polymorphisms; PON1; precision medicine

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INTRODUCTION

Clopidogrel, a second-generation thienopyridine antiplatelet drug, inhibits adenosine diphosphate (ADP)-induced platelet aggregation, acting through irreversibly binding of clopidogrel active metabolite (CAM) to the P2Y12 receptor. Currently, clopidogrel is used in combination with aspirin to prevent stent thrombosis after percutaneous coronary intervention (PCI) in patients with acute coronary syndrome (ACS). However, platelet responses to clopidogrel appear to be highly variable among individuals, and patients may be at an increased risk for major adverse cardiovascular events (also known as MACE) due to low response or therapy failure [1]. As illustrated in Figure 1, clopidogrel, as a prodrug, exerts its antiplatelet effects only after its metabolic activation. Clopidogrel requires a two-step oxidation, involving several enzymes such as CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, and paraoxonase (PON)-1, in which CYP2C19 plays an essential role but PON1 is responsible for the formation of endothiol metabolite in the second step oxidation [2–4].

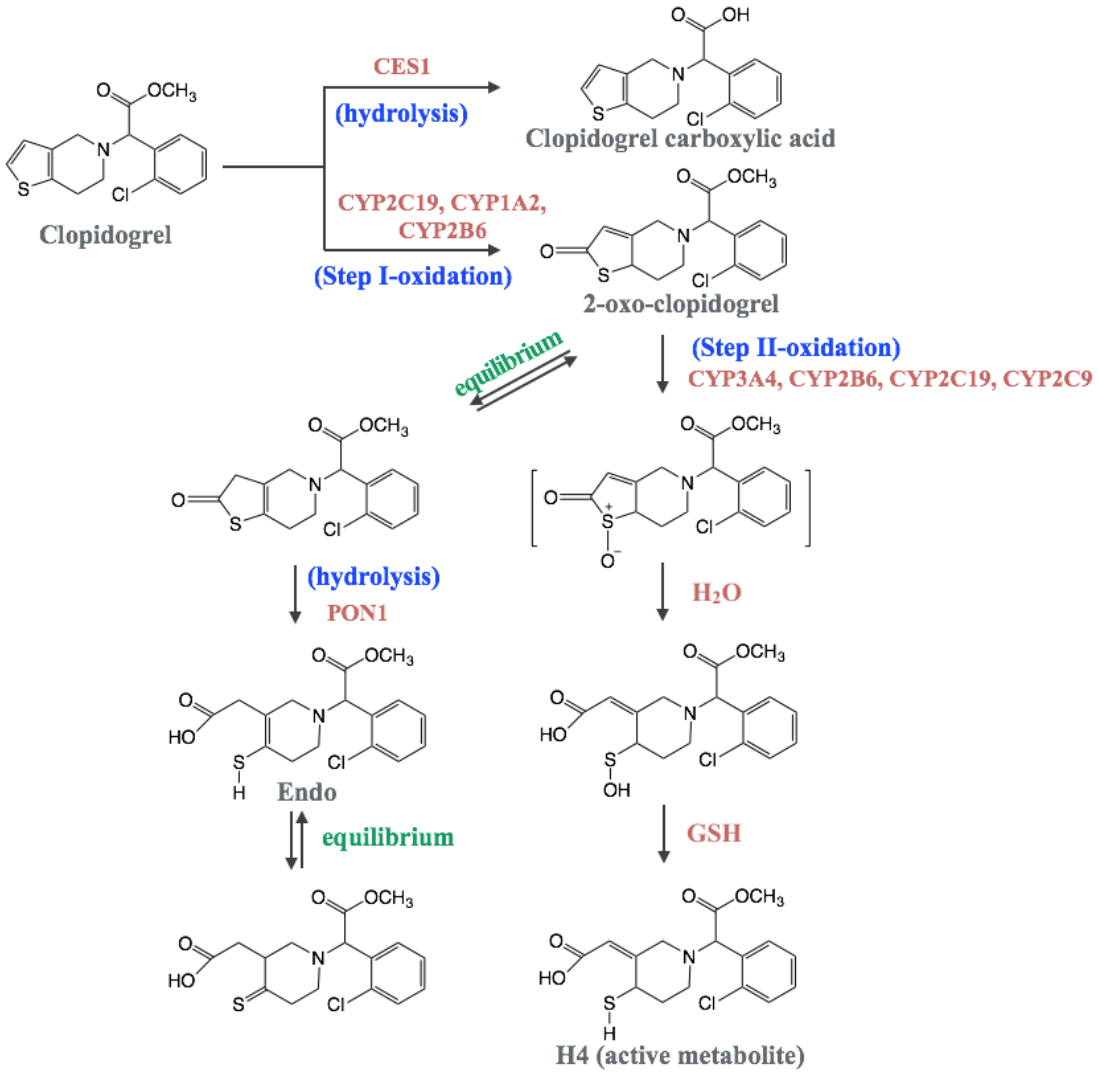

Figure 1. Proposed pathways involved in the formation of clopidogrel active metabolites H4 and endo in the metabolism of clopidogrel.

PON1 is an important member of the PON family, and the genes encoding the PONs are localized in tandem at the long arm of chromosome 7 ($7q^{21-22}$) in humans. PON1 is a 45 kDa glycoprotein, which is primarily synthesized in the liver and secreted into the blood. As a rate-limiting enzyme, PON1 hydrolyzes exogenous organophosphates (e.g., paraoxon, a toxic metabolite of insecticide parathion). PON1 is also associated with high-density lipoprotein (HDL), and functions as an antioxidant. In general, paraoxon (a probe substrate for PON activity) and phenylacetate (for arylesterase activity) are used to evaluate PON1 activity, respectively [5]. An earlier study published by Bouman et al. indicated that PON1 is the crucial enzyme for clopidogrel bioactivation in vitro [6]. Subsequently, several clinical research studies did not support such a finding [7–13]. Furthermore, in vitro evidence has proved that PON1 catalyzes the

formation of the minor thiol metabolite “endo”, which is different from the major thiol metabolite catalyzed by CYP450s [4].

Although PON1 itself does not seem to be a major determinant of clopidogrel efficacy, most of subsequent relevant clinical research studies were still concentrated on its association of PON1 genetic polymorphisms with clopidogrel platelet responses or clinical outcomes, with all the results conflicted. In this review article, we attempted to systematically summarize the role of PON1 in the metabolism of clopidogrel as well as the effect of the PON1 genetic polymorphisms on clopidogrel platelet response in patients treated with clopidogrel.

EFFECTS OF THE PON1 POLYMORPHISMS ON CLOPIDOGREL PLATELET RESPONSES AND CLINICAL OUTCOMES

The PON1 genetic polymorphisms

There are three single nucleotide polymorphisms (SNPs) in the promoter region of the PON1 gene, −108C>T (rs705379), −126C>G (rs705380), and −162A>G (rs705381). In its coding region, two SNPs or variants are L55M (163A>T, rs854560) and Q192R (575A>G, rs662) [14]. Of them, the most common is glutamine (Q) to arginine (R) change at the amino acid residues 192 (i.e., Q192R), which was observed to be associated with lower serum PON1 activity, impaired clopidogrel responses, and consequently increased risk of stent thrombosis in clopidogrel-treated patients. For example, the PON1 192Q/Q had the lowest PON1 activity and low rate of clopidogrel bioactivation in human liver microsome (HLM) [15−17]. Compared with the 192R allele, the 192Q allele with low PON1 activity was associated with a decrease in maximal concentrations of CAM and impaired inhibition of platelet aggregation in patients treated with clopidogrel [15]. However, carriers of the PON1 Q192 or R192 variant had similar effects on the MACE [6]. Also, the variant L55M was associated with altered expression level and serum activity of PON1 [18]. The genotype 55M/M was associated with the lower PON1 activity, but H4 levels and antiplatelet effects of CAM were not different from those of the 55L/L in acute myocardial infarction (AMI) patients treated with clopidogrel [8]. In its promoter region, polymorphism −108C>T affects PON1 promoter activity and enzyme expression [19]. Clearly, although the PON1 polymorphisms significantly affect the PON1 expression and its serum activity, most of the relevant clinical research studies have indicated that these genetic polymorphisms may not be associated with changes of either platelet response to clopidogrel or risk for the occurrence of MACE (see below for details).

Evidence for the PON1 genetic polymorphisms as a determinant of clopidogrel efficacy and clinical outcomes

An earlier study by Bouman et al. for the first time showed that PON1 was a key factor for clopidogrel bioactivation [6]. By using in vitro incubation, the R192 variant exhibited a higher hydrolysis activity for 2-oxo-clopidogrel than the Q192 (V_{max}/K_m, 1.36 vs. 0.36, $P < 0.001$). In the clinical research studies, the 192Q/Q was significantly correlated with lower plasma PON1 activity, lower C_{max} of CAM (approximately 5 times lower), lower platelet inhibition and higher risk for stent thrombosis compared with the 192R/R. Inparticular the contribution of the PON1 Q192R polymorphism to clopidogrel resistance variability accounted for 73%, but did not show an association of such individual variation with the CYP2C19 polymorphisms. Tselepis et al. verified the effects of the PON1 polymorphisms on platelet response and further demonstrated that both HDL levels and PON1 activity were correlated inversely with clopidogrel platelet activation in ACS patients undergoing PCI [15]. Despite the marked differences in ethnicity of patients and heterogeneity across disease status, the conclusions made from these studies performed in the patients of European descent were somehow consistent with those from East Asians and South Americans [16, 20−23].

Evidence against the PON1 genetic polymorphisms as a determinant of clopidogrel efficacy and clinical outcomes

On the contrary to the above, a large number of studies did not support any association of the PON1 genetic polymorphisms with clopidogrel response or MACE in most of the relevant clinical trials. Currently, few studies deciphered the true role of PON1 in clopidogrel bioactivation. Dansette et al. dissected the mechanisms underlying clopidogrel bioactivation, and elaborated the two metabolic pathways for the formation of opening thiolactone ring of 2-oxo-clopidogrel, with the major one catalyzed by CYP450 to form a “4b cis”, and the minor one by PON1 hydrolysis to form a “4b endo” [4] as illustrated in Figure 1.

Furthermore, only the "4b cis" diastereomers was found to be of clinical relevance [4]. Ancrenaz et al. confirmed the findings from Dansette et al. and further assessed the relative contribution of CYP450s and PON1 to clopidogrel metabolism in vitro, respectively [24]. The formation of CAM from 2-oxo-clopidogrel was the highest in HLM genotyped with CYP2C19*1/*1, followed by HLM with CYP2C19*2/*2, and human serum (500 times lower than HLM), whereas no CAM was detected in recombinant PON1 enzyme [24]. In addition, inhibition assay demonstrated that inhibitors of CYP3A, CYP2B6 and CYP2C19 significantly decreased clopidogrel metabolism, but that PON1 inhibitors, such as EDTA, exhibited little inhibition, indicating limited contribution of PON1 to clopidogrel bioactivation in vitro relative to the CYP450s involved [24]. Moreover, in a clinical study, Gong et al. indicated that CYP2C19 polymorphisms acted as a predictor for H4 levels and antiplatelet effects of clopidogrel, whereas serum PON1 activity did not [7]. The levels of "endo" metabolite formed from PON1 were extremely low in plasma, approximately 20 times lower than H4 levels, which could not be used to predict the antiplatelet effects [7]. Obviously, PON1 is only responsible for the formation of endothiol metabolite, rather than the formation of "4b cis" thiol metabolite, and thus plays a less important role in clopidogrel bioactivation than the CYP450s involved. Furthermore, the extremely low levels of endothiol metabolite in vivo is of less clinical relevance.

Subsequently, many clinical research studies have confirmed these findings, showing less contribution of the PON1 polymorphisms (Q192R or L55M) to clopidogrel responses and clinical outcomes, regardless of the disease patients suffered from (AMI, post MI, coronary arterial disease, acute ischemic stroke, transient ischemic attack, and more), the clinical outcomes assessed (MACE, death, MI, urgent coronary revascularization, stent thrombosis, and more) [8,9,11,12, 25–28], or the ethnicity of Asian patients (e.g., Han Chinese, Asian Indians, and Malaysians) [29–32]. Results of all relevant clinical studies are summarized in Table 1, in which CYP2C19 loss-of-function genotype is correlated well with the clopidogrel responses and with an increased risk for the adverse cardiovascular outcomes in patients treated with clopidogrel.

Study [ref.]	Patients	n	Ethnicity	*PON1* variant	Drug used	Platelet reactivity assay	Follow-up (mth)	Clinical outcome	Effects of *PON1* on clopidogrel response	Effect of *PON1* on clinical outcomes	Other related genetic or non-genetic factors
Bouman et al, 2011 [6]	SAP or CAD (PCI)	7719	white	Q192R	CLP	LTA	18	ST	Yes	Yes	-
Hulot et al, 2011 [8]	Post-MI	106	-	Q192R, L55M	CLP	LTA, VN	6	MACE	No	No	*CYP2C19*2
Lewis et al, 2011 [13]	PCI	566	white	Q192R	CLP	LTA	12	MACE	No	No	-
Sibbing et al, 2011 [12]	PCI	1524	-	Q192R	CLP	MEA	-	ST	No	No	*CYP2C19*2
Simon et al, 2011 [9]	AMI	2210	-	Q192R	CLP	-	12	MACE	-	No	*CYP2C19*2, *3
Trenk et al, 2011 [25]	PCI	760	-	Q192R	CLP	LTA, FCM	12	Death, MI	No	No	*CYP2C19*2. *17
Tselepis et al, 2011 [15]	ACS	74	-	Q192R	CLP	LTA, VASP	-	-	Yes	-	HDL level, PON1 activity
Chan et al, 2012 [29]	CAD (PCI)	89	Asian	Q192R	CLP	VASP	-	-	No	-	*CYP2C19*2, *3, *17
Chen et al, 2012 [32]	CAD (PCI)	4964	Chinese	Q192R, L55M, 108C>T	CLP	-	9	ST	-	No	*CYP2C19*2
Gong et al, 2012 [7]	Healthy CTL	21	-	Q192R	CLP	VN	-	-	No	-	*CYP2C19*2, *3
Kreutz et al, 2012 [33]	CAD	151	mixed	Q192R	DAT	LTA, VN	-	-	No	-	*CYP2C19*2
Pare et al, 2012 [10]	UAP	5059	white	Q192R	CLP	-	9	Composite death, nonfatal MI, stroke, MACE	No	No	-
Tang et al, 2012 [30]	PCI	670	Han Chinese	Q192R	DAT	TEG	12	CV death, nonfatal MI, TVR, ST	No	No	*CYP2C19*2, *3

Kang et al, 2013 [14]	CAD (PCI)	538 pts, 539 CTL	Han Chinese	108C > T , 126C > G, 162A > G, L55M, Q192R	DAT	-	12	MACE, bleeding events	-	Yes	*CYP2C19*2*
Li et al, 2013 [20]	UAP	180	Han Chinese	Q192R	CLP	LTA	-	-	Yes	-	*CYP2C19*2*
Nishio et al, 2013 [16]	PCI (DES)	112	Japanese	-	DAT	VN	9	intra-ST	Yes	Yes	PON1 activity
Park et al, 2013 [34]	PCI (DES)	1676	Korean	Q192R	CLP	VN	12	composite death, MI, ST	No	Yes	small dense LDL-C
Wu et al, 2013 [35]	ACS	424	Han Chinese	Q192R	CLP	TEG	-	-	Yes	-	*CYP2C19*2, *3
Zhang et al, 2013 [31]	ACS	500	Han Chinese	Q192R	CLP	LTA	-	-	No	-	carriers of at least one *CYP2C19* LOF
Martinez-Quintana et al, 2014 [36]	CAD	263	white	Q192R	DAT	-	12	ischemic event	No	No	-
Tresukosol et al, 2014 [11]	CAD	211	Thai	Q192R	DAT	MEA	-	-	No	-	*CYP2C19*2, *3, combined with smoking, diabetes, or increased platelet count
Chen et al, 2015 [22]	ACS (PCI)	336	Han Chinese	Q192R	DAT	TEG	6	MACE	Yes	Yes	*CYP2C19*2*
Mega et al, 2015 [26]	Healthy CTL and ACS pts	275, 2922	-	Q192R	CLP or PRAS	LTA	15	CV death, MI, stroke, ST	No	No	-
Nakkam et al, 2015 [37]	Healthy CTL	35	-	Q192R	CLP	WBI, VN	-	-	No	-	*CYP2C19*,CYP3A5 SNPs
Li et al, 2016 [21]	IS (ST)	268	Han Chinese	Q192R	DAT	-	12	Ischemic events	-	Yes	*P2Y12, COX1*
Marchini et al, 2017 [23]	Atherosclerotic (PCI)	187	-	Q192R	DAT	LTA	-	-	Yes	-	*CYP2C19*
Saydam et al, 2017 [19]	PCI	347	Turkish	Q192R, L55M	CLP	VN	-	-	No	-	*CYP2C19*2, *17
Zhang et al, 2017 [38]	PCI (DES)	136	Korean	Q192R	DAT	VN	-	-	No	-	*CYP2C19*2, *3

Table 1. The influence of the PON1 genetic polymorphisms on clopidogrel responses and clinical outcomes. ACS, acute coronary syndrome; AMI, acute myocardial infarction; CAD, coronary artery disease; CLP, clopidogrel; CTL (subjects), control; CV, cardiovascular; DAPT, dual antiplatelet therapy; DES, drug-eluting stent; FCM, flow cytometry; HDL, high-density lipoprotein; IS, ischemic stroke; LDL, low-density lipoprotein; LOF, loss-of-function; LTA, light transmission aggregometry; MACE, major adverse cardiovascular event; MEA, multiple electrode aggregometry; MI, myocardial infarction; mth, month; PCI, percutaneous coronary intervention; ref, reference; PRAS, prasugrel; pts, patients; SAP, stable angina pectoris; SNP, single nucleotide polymorphism; ST, stent thrombosis; TEG, thrombelastography; TVR, target vessel revascularization; UAP, unstable angina pectoris; VASP, vasodilator stimulated phosphoprotein; VN, VerifyNow; WBI, whole-blood impedance.

There are some causes that could result in conflicting conclusions for the effects of PON1 genetic polymorphisms on platelet responses to clopidogrel and its clinical outcomes. First of all, accurate and stereoselective quantification of H4 may be the major cause. CAM consists of four isomers, in which only the inactive H3 and active H4 can be detected in human plasma. Therefore, a highly stereoselective LC-MS/MS method is required to quantify levels of H4 and other active metabolite isomers in plasma [39,40]. Second, CAM is unstable and therefore, its rapid derivatization by alkylate agents (e.g., MPB) is required to stabilize the free thiol metabolite [40]. However, in the study by Bouman et al. [6], an alternative method was used for such stabilization, which might lead to the discrepant results. Third, the standard chemicals

of thiol metabolite obtained from Bouman et al. were obtained by purifying of PON1-mediated 2-oxo-clopidogrel, which may not be entirely accurate. Forth, the concentrations of clopidogrel and its intermediate metabolite used for in vitro incubation assays should be clinically relevant. As an intermediate metabolite, plasma concentrations of 2-oxo-clopidogrel were extremely low. However, Bouman et al. used significantly higher substrate concentrations [6]. And last but not least importantly, Bouman et al. indicated that the PON1 polymorphisms contributed 73% for clopidogrel response variability whereas CYP2C19 did not [6], which was totally different from the almost all previous clinical studies, and a recent genome-wide association study [41]. A possible reason may be inconsistently heterologous expression systems used in different studies. In addition to the above reasons, other factors, such as baseline characteristics of the enrolled patients, concomitant use of drugs, and CYP2C19 genotype, could cause inconsistent results.

CONCLUSSIONS

PON1 has emerged as an important pharmacogenetics regulator of clopidogrel bioactivation in the past [6,15,16,20–23]. However, this finding was not confirmed by a large number of clinical research studies [4,7–13,24–32]. This mini-review article demonstrated that the influence of the PON1 genetic polymorphisms on clopidogrel bioactivation and clinical outcomes was limited. Many factors may result in such conflicting conclusions, including methodologies used and patients enrolled. Considering the presence of potential gene-gene interactions, only carriers of the CYP2C19*1/*1 genotype would be chosen to explore the true effect of PON1 and its genetic polymorphisms on the bioactivation of and response to clopidogrel. If PON1 is not confirmed to be responsible for the metabolism of clopidogrel in vitro, in particular after use of recombinant PON1 alone or in combination with highly specific chemical inhibitors of or monoclonal antibody against PON1, the influence of the PON1 genetic polymorphisms on clopidogrel bioactivation in vivo would be of less clinical relevance. In addition to clopidogrel, next-generation antiplatelet drugs, such as prasugrel and vicagrel, were designed to significantly increase the efficiency of the formation of 2-oxo-clopidogrel (an immediate precursor of CAM) in the intestine by carboxylesterase 2-mediated hydrolysis, acting through bypassing the first-step P450-mediated oxidation of clopidogrel in the liver [42–46]. It is concluded that PON1 would also play a less important role in the metabolism of these novel antiplatelet drugs than CYP450s and carboxylesterase 2 involved.

CONFLICT OF INTEREST

The author declares no competing interests.

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FINANCIAL DISCLOSURE

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