European Heart Journal (2004) **25**, 1903–1910

Clinical research

High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability

Dominick J. Angiolillo[a,*], Antonio Fernández-Ortiz[b], Esther Bernardo[b], Celia Ramírez[b], Manel Sabaté[b], Camino Bañuelos[b], Rosana Hernández-Antolín[b], Javier Escaned[b], Raul Moreno[b], Fernando Alfonso[b], Carlos Macaya[b]

[a] *Division of Cardiology, University of Florida, Shands Jacksonville, 655 West 8th Street, ACC Building, Jacksonville, FL 32209, USA*
[b] *Cardiovascular Institute, San Carlos, University Hospital, Madrid, Spain*

Received 29 April 2004; revised 26 July 2004; accepted 29 July 2004

KEYWORDS
Copidogrel;
Coronary stenting;
Platelet function

Aim To assess platelet inhibitory effects, interindividual variability in platelet inhibition as well as response to a 600 mg, compared to a standard 300 mg, clopidogrel loading dose (LD) after coronary stenting.
Methods and results Platelet function profiles were assessed in 50 patients undergoing coronary stenting receiving either a 300 mg (n = 27) or 600 mg clopidogrel LD. ADP (6 μM) and collagen (6 μg/mL) induced platelet aggregation, as well as ADP (2 μM) induced glycoprotein (GP) IIb/IIIa activation and P-selectin expression were assessed at baseline and 4, 24, and 48 h following clopidogrel front-loading. A more intense and rapid inhibition of platelet activation (both GP IIb/IIIa activation and P-selectin expression) were achieved using a 600 mg, compared to a 300 mg, LD throughout the entire 48 hours ($p < 0.001$). Although there were no differences in platelet aggregation, overall a 600 mg LD increased the number of clopidogrel responders and this was also achieved earlier compared to a 300 mg LD. A 600 mg LD did not reduce interindividual variability of platelet response.
Conclusion The use of a 600 mg clopidogrel LD in patients undergoing coronary stenting optimises platelet inhibitory effects early after intervention and may provide a more effective protection against early thrombotic complications.
© 2004 Published by Elsevier Ltd on behalf of The European Society of Cardiology.

Introduction

The combination of aspirin and clopidogrel represents the treatment of choice to prevent stent thrombosis early after coronary stenting (CS).[1] Clopidogrel has a better safety profile and a more accelerated antiplatelet activity than ticlopidine using a standard 300 mg oral loading dose (LD).[2–6] Such rapid antiplatelet effects obtained with clopidogrel front loading is crucial since thrombotic complications commonly occur within the first 24–48 h following CS.[1] However, thrombotic events

* Corresponding author. Tel.: +1-904 2443933; fax: +1-904 2443102.
E-mail address: dominick.angiolillo@jax.ufl.edu (D.J. Angiolillo).

0195-668X/$ - see front matter © 2004 Published by Elsevier Ltd on behalf of The European Society of Cardiology.
doi:10.1016/j.ehj.2004.07.036

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

still occur despite the use of dual antiplatelet treatment and this may, at least in part, be attributed to an inadequate effect of antiplatelet therapy.[7]

Recent data have demonstrated an interindividual variability of platelet inhibition in patients undergoing CS receiving a standard 300 mg clopidogrel LD on top of aspirin.[8–9] In particular, approximately one-third of patients may have a suboptimal antiplatelet response early after intervention using this treatment regimen.[7–11] Although clopidogrel response improves over time,[8] a high clopidogrel LD regimen has been suggested to optimise this response more rapidly. However, the effect of a high clopidogrel LD on platelet reactivity, interindividual variability of platelet inhibition and number of clopidogrel responders has still not been well defined. Thus, the aim of this study was to compare platelet function profiles in the early hours after coronary stent intervention in patients receiving a standard 300 mg clopidogrel LD with that obtained following a 600 mg LD and to assess interindividual variability in platelet inhibition as well as the number of clopidogrel responders/non-responders.

Methods

Patient population and study protocol

Fifty patients referred to our interventional cardiology unit for elective coronary stenting were included: 27 patients received a 300 mg clopidogrel LD and 23 received a 600 mg LD. Allocation to one or the other treatment group was consecutive: the first 27 patients received a 300 mg LD while the final 23 patients were assigned to a 600 mg LD. Clopidogrel loading was administered immediately after intervention. Clopidogrel (75 mg/daily) was then administered with 24-h intervals during hospitalisation and maintained for one month. All patients were on aspirin (250 mg/daily) for at least 7 days before procedure. During the procedure, unfractioned heparin (100 IU/kg) was administered and activation clotting time values were maintained above 250 s. Only commercially available bare metal stents were used. Exclusion criteria were use of lysis therapy or platelet glycoprotein (GP) IIb/IIIa blockers before, during, or after intervention; prior use of thienopyridines; and/or aspirin intolerance/allergy. Blood samples for platelet function assays were collected before intervention (baseline sample) whilst patients were only on aspirin and then 4, 24, and 48 h following clopidogrel administration. Blood was drawn from a 6-French arterial sheet for baseline and 4-h samples and from an antecubital vein using a 21-gauge needle for the 24- and 48-h samples. The initial first millilitres were discarded to avoid spontaneous platelet activation. Platelet count was assessed at all time points to ensure that the degree of platelet reactivity was not biased by the number of platelets in each group. Patients were not included if platelet counts were outside the range of 125–450 10^9/L.

This study complied with the Declaration of Helsinki, was approved by the Ethical Committee of the San Carlos University Hospital and all patients gave their informed consent.

Assessment of platelet aggregation

Blood was collected in tubes containing 3.8% trisodium citrate for assessment of platelet aggregation. Aggregation induced by 6 μM ADP (Chrono-Log Corp., Havertown, PA, USA) and 6 μg/mL collagen (Chrono-Log Corp., Havertown, PA, USA) was assessed in platelet-rich plasma (PRP) using the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, PA, USA).[12] Agonists (type and concentration) were used as previously reported.[13] PRP was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 min. The platelet count in PRP was adjusted to the range of 150,000–300,000/L by dilution with autologous plasma when out of range. The isolated PRP was kept at 37 °C before use. Platelet poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2500 rpm for 10 min. Light transmission was adjusted to 0% with PRP and to 100% for PPP in each measurement. Platelet aggregation was assessed within 2 h from blood sampling. Curves were recorded for 5 min and platelet aggregation was determined as the maximal percent change in light transmittance from baseline using PPP as reference.

Assessment of platelet activation

Platelet activation was determined by assessing platelet surface expression of activated GP IIb/IIIa and P-selectin following 2 μM ADP (ChronoLog, Havertown, PA) stimuli through flow cytometry as previously described.[14–15] Whole blood was drawn into sterile tubes containing 3.8% trisodium citrate and then diluted with Hepes–tyrodes buffer (5 mM Hepes [hydroxyethylpiperazine-ethane-sulfonic acid], 137 mM NaCl, 2.7 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 2 mM $CaCl_2$, 5 mM glucose, 0.2% BSA) to a final volume of 1:8:1 (blood: Hepes–tyrodes: citrate) resulting in a 1/10 dilution during sampling. Then, 50 μL of diluted blood was stimulated with 2 μM ADP before immunolabelling. GP IIb/IIIa activation was assessed using a polyclonal fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human fibrinogen antibody (800 nM, DAKO Diagnostics, Glostrup, Denmark) to detect fibrinogen binding to the activated GPIIb/IIIa complex. Similarly, P-selectin expression was assessed using a phycoerythrin (PE)-conjugated anti-CD62P (0.3 mg/ml, Becton Dickinson, San José, CA, USA). Twenty minutes after incubation with the corresponding antibody, 300 μl of 0.5% PBS-buffered paraformaldehyde was added for fixation. Samples were analysed within 2 h on an EPICS-XL PROFILE II Coulter flow cytometer (Coulter Corp. Miami, FL, USA). Light scatter and fluorescence data from 10,000 platelet events were collected with all detectors in logarithmic mode. Platelet activation was expressed as the percentage of platelets positive for antibody binding. Non-specific immunostaining for rabbit anti-human fibrinogen was determined using an irrelevant isotype IgG-FITC conjugated, purified from normal rabbit serum, and non-specific immunostaining for CD62P was determined using an irrelevant isotype IgG-PE conjugated (Becton Dickinson, San José, CA, USA), and thus served as negative controls.

Definition of clopidogrel response

Response to clopidogrel treatment was defined according to the degree of inhibition of platelet function (platelet aggregation, GP IIb/IIIa activation and P-selectin expression) after clopidogrel administration compared with baseline values (before clopidogrel). In line with previous reports,[7,10] patients were classified as non-responders, low responders, and responders when platelet inhibition was <10%, 10–29%, and $\geqslant$30%, respectively.

Statistical analysis

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequencies and percentages. Comparisons between categorical variables were performed using

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

the two-tailed Fisher's exact test or Pearson's χ^2 test as appropriate. The Student's *t*-test was used to compare continuous variables as these were normally distributed. A multivariate analysis of variance (MANOVA) with Bonferroni correction was used to assess differences between groups during the overall study time course. A p value <0.05 was considered statistically significant. The coefficient of variability (CV) was used to assess the interindividual variability in each of the platelet function assays (CV = SD/mean). A significant variability was defined when the CV was higher than 0.25 in continuous variables with a normal distribution. Statistical analysis was performed using a SPSS v11.0 software (SPSS Inc. Chicago, IL, USA).

Results

Characteristics of the study population

Baseline demographic, angiographic and biological data are provided in Tables 1 and 2. All patients had a platelet count within the pre-defined range. Coronary stenting was successfully performed in all patients and no bleeding complications occurred.

Platelet aggregation

ADP- and collagen-induced platelet aggregation was significantly reduced respect to baseline at 4, 24, and 48 h after 300 and 600 mg clopidogrel LD ($p < 0.01$ for all comparisons respect to baseline). Platelet aggregation profiles during the entire study period were similar using 6 μM ADP (MANOVA; $p = 0.84$) and 6 μg/mL collagen (MANOVA; $p = 0.48$) between both treatment groups (Fig. 1(a) and (b)).

GP IIb/IIIa activation

GP IIb/IIIa activation was significantly reduced with respect to baseline at 4, 24, and 48 h in both treatment groups ($p < 0.05$ for all comparisons with respect to baseline). A greater inhibition of ADP-induced GP IIb/IIIa activation was achieved in patients receiving a 600 mg, compared to a 300 mg, clopidogrel LD 4 hours after drug administration and significant differences were maintained at 24 and 48 h (Fig. 2(a)). GP IIb/IIIa activation was significantly lower during the overall study time course in the high LD group (MANOVA; $p = 0.001$; Fig. 2a). Following a 600 mg clopidogrel LD, platelet inhibition of GP IIb/IIIa activation was 46% greater than that achieved with a 300 mg LD as early as 4 h after intervention. Afterwards, a stable degree of inhibition of the GP IIb/IIIa receptor persisted at 24 and 48 h following a 600 mg clopidogrel loading, whilst inhibition of the GPIIb/IIIa receptor gradually increased in the 300 mg group at 24 and 48 h. At 48 h after treatment, patients receiving a 600 mg loading still had a greater inhibition of GP IIb/IIIa activation compared to those treated with a standard 300 mg LD ($p = 0.02$; Fig. 2(a)).

P-selectin expression

P-selectin expression significantly reduced with respect to baseline at 4, 24, and 48 h in both treatment groups ($p < 0.05$ for all comparisons respect to baseline). As observed for GPIIb/IIIa activation, a greater inhibition of ADP-induced P-selectin expression was achieved in patients receiving a 600 mg compared to a 300 mg clopidogrel LD at 4, 24, and 48 h and P-selectin expression was significantly lower during the overall study time course in the high LD group (MANOVA; $p = 0.001$; Fig. 2(b)).

Table 1 Demographics of the study population

	300 mg ($n = 27$)	600 mg ($n = 23$)	p
Age (years)	61 ± 11	62 ± 11	0.68
Male	22 (81%)	19 (83%)	1.00
Risk factors			
Smoking habitus	8 (30%)	4 (17%)	0.34
Hyperlipidaemia	17 (63%)	19 (83%)	0.20
Diabetes	6 (22%)	3 (13%)	0.47
Hypertension	16 (59%)	13 (56%)	1.00
Prior myocardial infarction	17 (63%)	13 (56%)	0.77
Prior percutaneous coronary intervention	6 (22%)	7 (30%)	0.53
Prior aortocoronary bypass surgery	—	2 (9%)	0.20
Prior cerebrovascular event	1 (4%)	—	1.00
Peripheral vascular disease	3 (13%)	2 (7%)	0.65
Treatment			
β-Blockers	20 (74%)	12 (52%)	0.14
Nitrates	16 (59%)	13 (56%)	1.00
ACE-inhibitors	8 (30%)	4 (17%)	0.34
Statins	15 (56%)	16 (70%)	0.38
Calcium-blockers	7 (26%)	6 (26%)	1.00

Values are expressed as means ± SD and absolute number (percentages).

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

Table 2 Angiographic and biological data of the study population

	300 mg (n = 27)	600 mg (n = 23)	p
Angiographic data			
LAD	12 (44%)	9 (39%)	0.77
LCx	7 (26%)	5 (22%)	1.00
RCA	15 (56%)	14 (61%)	0.77
Multi-vessel Stenting	6 (22%)	5 (22%)	1.00
Number of stents	1.3 ± 0.6	1.4 ± 0.6	0.52
Stent size (mm)	3.1 ± 0.4	3.2 ± 0.5	0.58
Stent length (mm)	26.5 ± 18.1	26.6 ± 12.3	0.98
B2-C Lesions	25 (67%)	18 (64%)	0.72
Platelet count			
Baseline (10^9/L)	187.4 ± 60.8	207.8 ± 61.7	0.24
4 hours (10^9/L)	187.8 ± 52.3	177.7 ± 46.2	0.52
24 hours (10^9/L)	199.2 ± 70.1	190.0 ± 51.0	0.63
48 hours (10^9/L)	191.2 ± 51.5	192.2 ± 46.2	0.95

LAD: left anterior descending coronary artery; LCx: left circumflex coronary artery; RCA: right coronary artery; B2-C lesions: complex coronary lesions according to the American College of Cardiology/American Heart Association grading system. Values are expressed as absolute number (percentages) and means ± SD.

Clopidogrel response and interindividual variability

Although the number of responders varied according to the platelet function assay used for its definition, overall a 600 mg LD was associated with a higher number of clopidogrel responders (Table 3). In particular, the number of responders was significantly higher at 4 and 24 h for inhibition of GP IIb/IIIa activation and at 4 and 48 h for P-selectin inhibition. A significantly higher number of clopidogrel responders were observed at 24 and 48 h for collagen-induced platelet aggregation and at 24 h for ADP-induced platelet aggregation. Importantly, a higher number of responders was achieved earlier (at 4

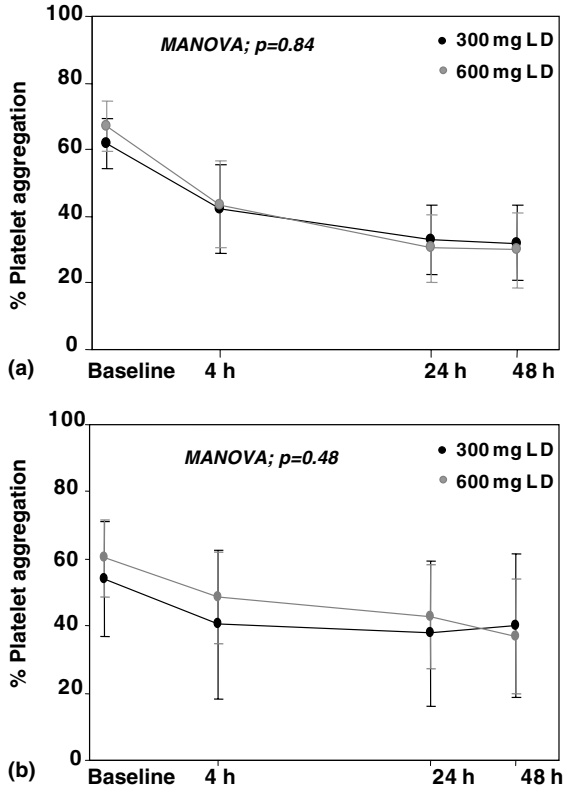

Fig. 1 Platelet aggregation following 6 μM ADP stimuli (a) and 6 μg/mL collagen stimuli (b). MANOVA= multivariate analysis of variance.

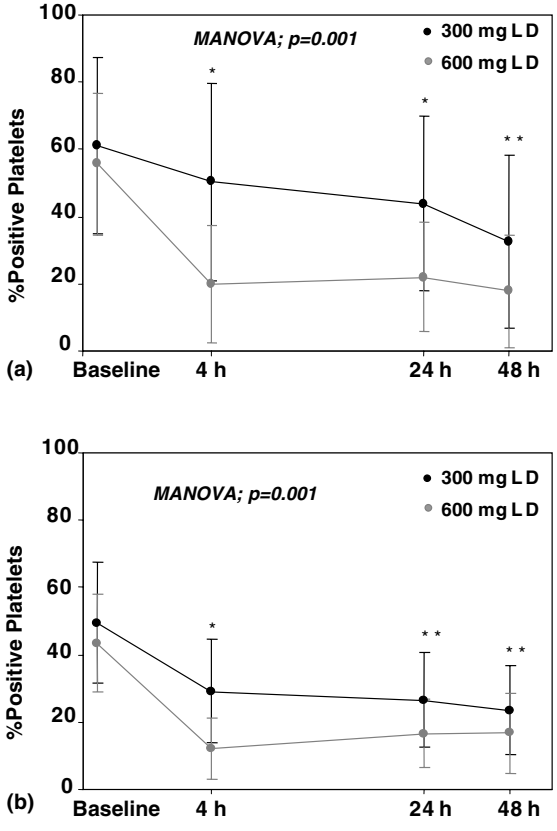

Fig. 2 GPIIb/IIIa activation (a) and P-selectin expression (b) following 2 μM ADP stimuli. *p < 0.001 and **p < 0.05 for intergroup comparison for given study time point; MANOVA = multivariate analysis of variance.

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

h) using a high LD and subsequently a minor increase in the number of clopidogrel responders over the study time period was seen in this group. Using a 300 mg LD, the number of clopidogrel responders progressively continued to increase over time. Interestingly, despite the greater degree of platelet inhibition, and thus of clopidogrel responders, which was achieved earlier with a high LD regimen, a significant interindividual variability of platelet inhibition persisted (Fig. 3).

Discussion

The main result of this study is that a 600 mg clopidogrel LD is able to achieve a more intense and rapid inhibition of platelet activation (ADP-induced GP IIb/IIIa activation and P-selectin expression) compared to a standard 300 mg LD during the first 48 h after coronary stent implantation. This higher clopidogrel front loading regimen increased the number of clopidogrel responders and this was also achieved earlier compared to a 300 mg LD regimen. Importantly, despite an overall improvement in clopidogrel response, an increased front loading was not associated with a reduction in the interindividual variability in clopidogrel induced platelet inhibition.

The rationale for using a 300 mg clopidogrel LD in clinical practice derives from dose finding studies performed mainly in healthy volunteers.[3,6] Platelet reactivity in patients with coronary atherosclerosis undergoing coronary stenting may be significantly different from that of healthy individuals. In fact, in this clinical setting there is an increased thrombotic *milieu* not observed in healthy individuals due to several factors, such as the presence of primed platelets[15] or increased platelet reactivity induced by coronary stenting[8,9] and/or heparin administration.[16] In addition, drug-drug interactions inhibiting clopidogrel activation by the hepatic cytochrome P450 (CYP) 3A4,[17] genetic polymorphisms (GPIIb/IIIa receptor, GPIa receptor, $P2Y_{12}$ receptor),[15,18,19] and signalling defects may also modulate clopidogrel-induced antiplatelet effects. Therefore, there is a large degree of evidence suggesting that a 300 mg LD may not be sufficient to inhibit platelet reactivity leading to a significant proportion of patients having a poor clopidogrel response. Although we cannot assume that poor responders to a 300 mg clopidogrel LD from our study would have had a better response with a 600 mg LD (a cross-over study design would be best to address this issue), a high LD regimen appear as a simple and attractive solution. Our results using a 600 mg LD demonstrate an improved clopidogrel response in the early hours following coronary intervention, even if a significant degree of interindividual variability in platelet inhibition persists. The high number of factors contributing to modulation of platelet function in patients undergoing coronary interventions may explain the persistence of interindividual variability in platelet inhibition observed following treatment with a higher clopidogrel LD or even with more potent antiplatelet drugs.[20]

High clopidogrel LD regimens have already been tested in several coronary intervention studies, both in low[21] and in high risk settings,[22] and have been shown to be safe and potentially beneficial. However, functional data comparing different LD regimens are limited.

Table 3 Clopidogrel responsiveness (300 vs. 600 mg)

	4 h		24 h		48 h	
	300 mg (n = 27)	600 mg (n = 23)	300 mg (n = 27)	600 mg (n = 23)	300 mg (n = 27)	600 mg (n = 23)
ADP						
Non-responders	7 (25.9)	4 (17.4)	3 (11.1)	1 (4.3)	2 (7.4)	0 (0)
Low responders	7 (25.9)	2 (8.6)	5 (18.5)	0 (0)	3 (11.1)	1 (4.3)
Responders	13 (48.1)	17 (73.9)	19 (82.5)	22 (95.7)	22 (81.5)	22 (95.7)
p	0.15		0.05		0.36	
Collagen						
Non-responders	11 (40.7)	6 (21.7)	13 (48.1)	4 (17.4)	11 (40.7)	3 (13.0)
Low responders	7 (25.9)	7 (30.4)	2 (7.4)	7 (30.4)	7 (25.9)	3 (13.0)
Responders	9 (33.3)	10 (43.5)	12 (44.4)	12 (52.2)	9 (33.3)	17 (74.0)
p	0.54		0.03		0.015	
GPIIb/IIIa activation						
Non-responders	10 (37.0)	3 (13.0)	10 (37.0)	3 (13.0)	3 (11.1)	2 (8.7)
Low responders	4 (14.8)	1 (4.3)	5 (18.5)	1 (4.3)	4 (14.8)	2 (8.7)
Responders	13 (48.1)	19 (82.6)	12 (44.4)	19 (82.6)	20 (74.1)	19 (82.6)
p	0.04		0.02		0.75	
P-selectin						
Non-responders	4 (14.8)	0 (0)	4 (14.8)	0 (0)	9 (39.1)	0 (0)
Low responders	6 (22.2)	1 (4.3)	5 (18.5)	2 (8.7)	6 (22.2)	3 (13.0)
Responders	17 (63.0)	22 (95.7)	18 (66.7)	21 (91.3)	12 (44.4)	20 (86.7)
p	0.02		0.07		0.003	

Values are expressed as absolute number (percentages) and compared by Pearson's χ^2 test.

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

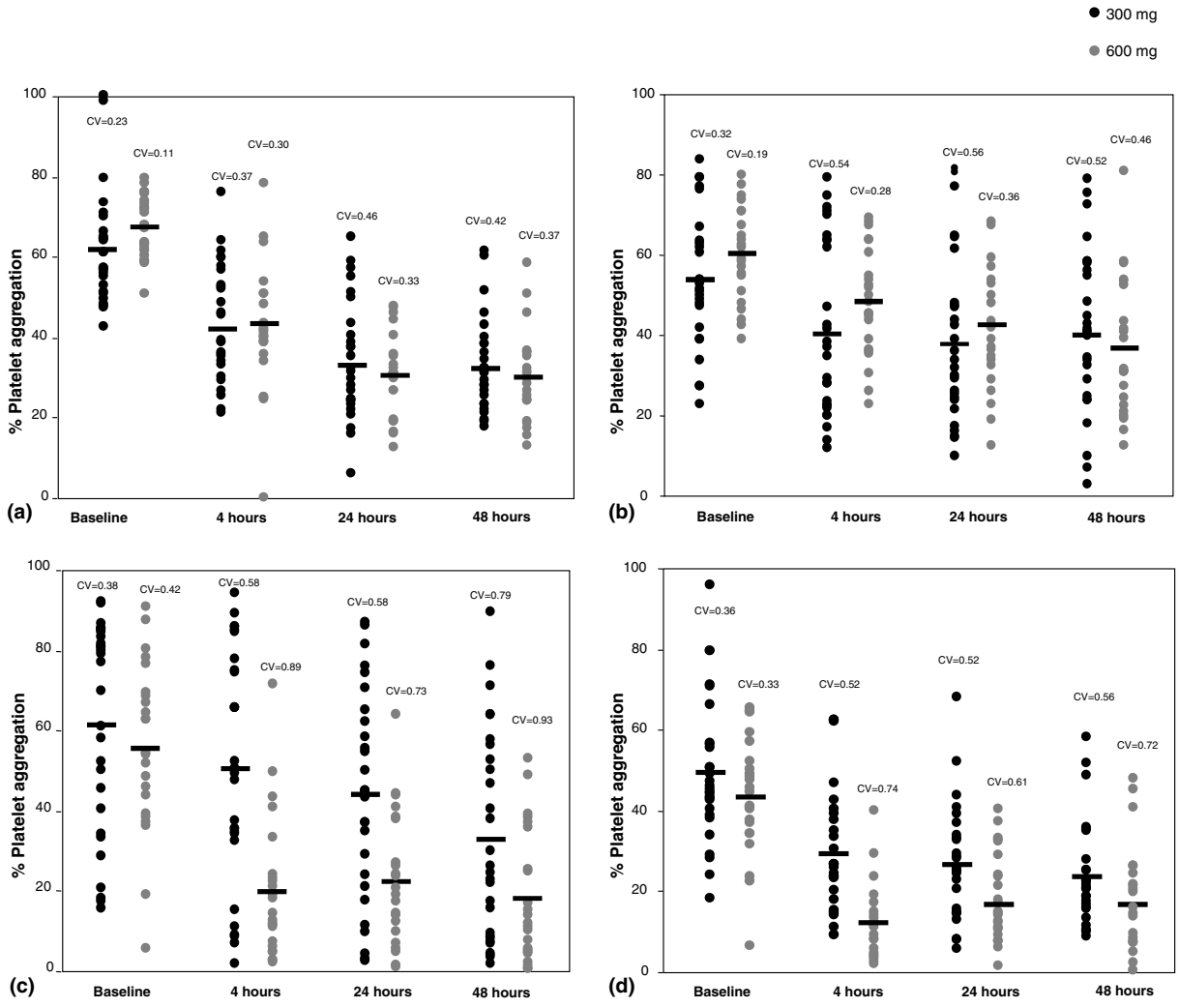

Fig. 3 Interindividual variability in 6 μM ADP-induced platelet aggregation (a), 6 μg/mL collagen-induced platelet agreggation (b), 2 μM ADP-induced GPIIb/IIIa activation (c), and 2 μM ADP-induced P-selectin expression (d). CV = coefficient of variability (CV = SD/mean; CV > 0.25 significant variability).

Muller et al.[23] observed that a 600 mg clopidogrel LD significantly reduced ADP-induced platelet aggregation more than a 300 mg LD and more than a ticlopidine 2×500 mg LD (10 patients per group) up to 48 h after drug administration. These authors also observed that P-selectin expression was only reduced in the higher clopidogrel LD group. Seyfarth et al.[24] observed a greater inhibition of ADP-induced P-selectin expression in 11 patients receiving a 450 mg clopidogrel LD compared to 21 patients receiving a 300 mg clopidogrel LD at 24 and 48 h following drug administration. Importantly, inhibition of P-selectin expression, as that also observed in our study, supports clopidogrel's potential anti-inflammatory effects and may represent an additional mechanism through which clopidogrel provides clinical benefit.[25] In addition to these data, our study also measured GP IIb/IIIa activation, the final mediator of platelet aggregation, upon which a 600 mg clopidogrel LD achieves a more rapid and intense inhibition than a 300 mg LD. Furthermore, our results also show that a high LD is able to increase the number of clopidogrel responders although interindividual variability in platelet inhibition remains high.

Clinical implications

Previous studies have demonstrated that clopidogrel pretreatment (at least 6 h) in patients undergoing coronary interventions is associated with a better one-month outcome.[26] In a subanalysis from the Clopidogrel for the Reduction of Events During Observation (CREDO) trial such benefit was not achieved unless pre-treatment was initiated at least 15 h before intervention. Since in the ''real world'' coronary interventions are frequently being performed immediately after diagnostic angiography, a clopidogrel pre-treatment strategy within the suggested time frame is frequently not feasible. Therefore, there is still a need to optimise the degree of platelet inhibition early after interventions, especially in high risk settings (acute coronary syndromes), in order to reduce

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

early thrombotic risk and a 600 mg clopidogrel LD at the time of intervention may safely be used to reach this goal.[12,23,27]

Although the most rapid inhibition of platelet function is achieved using intravenous GP IIb/IIIa inhibitors, data from the Intracoronary Stenting and Antithrombotic Regimen-Rapid Early Action for Coronary Treatment (ISAR-REACT) trial demonstrate that low and intermediate risk patients undergoing elective PCI receiving a 600 mg clopidogrel LD at least 2 h before intervention had the same one month clinical outcome as patients receiving abciximab in addition to a 600 mg clopidogrel LD.[12] Importantly, this was associated with a more favourable per se safety profile and outcome did not improve with the number of hours of pre-treatment. The data from our study demonstrate that a 600 mg clopidogrel LD induces a rapid and marked inhibition of platelet reactivity in patients undergoing elective stenting. This may help explain why there was a lack of additional benefit obtained with the adjunct use of abciximab in the relatively low risk patient population from the ISAR-REACT trial.

Study limitations

The present study has same potential limitations. First, the definition of an optimal response to clopidogrel treatment is empiric. However, the definition that we adapted is in line with that suggested in previous reports[7,10] and is reliable to identify relative differences in platelet inhibition between the two treatment strategies. Although the clinical implications for clopidogrel response still needs verification, it is well known from previous reports that ischaemic events are associated with increased platelet reactivity and suboptimal antiplatelet response.[21,28] Therefore, even if the small sample size of the study did not allow clinical differences (i.e., stent thrombosis) to be found, our findings are still relevant because a 600 mg clopidogrel LD reduced platelet activation to a greater extent and more rapidly than a 300 mg LD.

Second, this was not a randomized study. However, patients were consecutively allocated to one treatment arm (300 mg) and afterwards to the other (600 mg), limiting study bias. Importantly, the two treatment groups were also homogeneous for clinical presentation, risk factors, treatment and procedural data (Tables 1 and 2). Furthermore, lipophilic statins, highly debated to be involved in inhibiting clopidogrel activation through interaction with the hepatic CYP3A4 enzyme, [18] were equally distributed in the two treatment groups (7/15 and 8/16 statin treated patients in the 300 and 600 mg clopidogrel LD groups, respectively) and, in accordance with previous reports,[11,29,30] we did not observe reduction of the antiplatelet efficacy of clopidogrel (data not shown).

Ultimately, unlike some reports,[23] in our study a 600 mg clopidogrel LD failed to demonstrate differences in platelet aggregation when compared to a 300 mg LD. Although this may be related to the concentration of agonists used in our study (whereas higher concentrations may have unmasked underlying differences between the two treatment regimens), our data are in accordance with previous findings in which a high clopidogrel LD regimen (450—600 mg) achieved a similar degree of platelet aggregation using both a low and high agonist concentration.[31] Furthermore, the consistency of our results with two different agonists (ADP and collagen) used to induce platelet aggregation in vitro, and the overall greater number of clopidogrel responders observed with other assays in our study in patients receiving a high LD, support the validity of our findings.

Conclusions

The use of a 600 mg clopidogrel LD regimen induces a more enhanced platelet inhibition than a 300 mg LD in the early hours after coronary stenting. This increases and accelerates the number of clopidogrel responders and may therefore reduce the risk of early, acute thrombotic complications. A high clopidogrel LD regimen does not overcome interindividual variability of platelet inhibition, suggesting that other factors besides drug dose may still be involved in this phenomenon. Larger studies are warranted to assess the clinical impact of higher clopidogrel LDs compared to a standard 300 mg approach in order to define the optimal clopidogrel front-loading regimen in the setting of percutaneous coronary revascularization.

Acknowledgements

Dominick J. Angiolillo was supported by the Marie Curie Individual Fellowship. This work has been partially supported by a Grant from the Spanish Ministry of Health (FIS 00/0026-1).

References

1. Moussa I, Oetgen M, Roubin G et al. The effectiveness of clopidogrel and aspirin versus ticlopidine and aspirin in preventing stent thrombosis after coronary stent implantation. *Circulation* 1999; **99**:2364—6.
2. CAPRIE Steering Committee. A randomised, blinded trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996;**348**:1329—39.
3. Cadroy Y, Bossavy JP, Thalamas C et al. Early potent antithrombotic effect with combined aspirin and LD of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation* 2000;**101**:2823—8.
4. Helft G, Osende JI, Worthley SG et al. Acute antithrombotic effect of front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin. *Arterioscler Thromb Vasc Biol* 2000;**20**:2316—21.
5. Gawaz M, Seyfarth M, Muller I et al. Comparison of effects of clopidogrel versus ticlopidine on platelet function in patients undergoing coronary stent placement. *Am J Cardiol* 2001;**87**:332—6.
6. Savcic M, Hauert J, Bachmann F et al. Clopidogrel LD regimens:kinetic profile of pharmacodynamic response in healthy subjects. *Semin Thromb Hemost* 1999;**25**(Suppl. 2):15—9.
7. Muller I, Besta F, Schulz C et al. Prevalence of clopidogrel nonresponders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003; **89**:783—7.
8. Gurbel PA, Bliden KP, Hiatt BL et al. Clopidogrel for coronary stenting:response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;**107**:2908—13.

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022

9. Gurbel PA, Cummings CC, Bell CR et al. Onset and extent of platelet inhibition by clopidogrel loading in patients undergoing elective coronary stenting:the Plavix Reduction Of New Thrombus Occurrence (PRONTO) trial. *Am Heart J* 2003;**145**:239–47.
10. Lau WC, Gurbel PA, Watkins PB et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation* 2004;**109**:166–71.
11. Lepantalo A, Virtanen KS, Heikkila J et al. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. *Eur Heart J* 2004;**25**:476–83.
12. Ruggeri ZM. New insights into the mechanisms of platelet adhesion and aggregation. *Semin Hematol* 1994;**31**:229–39.
13. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al. Is a 300 mg clopidogrel LD sufficient to inhibit platelet function early after coronary stenting? A platelet function profile study. *J Invasive Cardiol* 2004;**16**:325–9.
14. Hagberg IA, Lyberg T. Blood platelet activation elevated by flow cytometry:optimised methods for clinical studies. *Platelets* 2000;**11**: 137–50.
15. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al. Pl^A polymorphism and platelet reactivity following clopidogrel LD in patients undergoing coronary stent implantation. *Blood Coagul Fibrinol* 2004;**2**:89–93.
16. Knight CJ, Panesar M, Wilson DJ et al. Increased platelet responsiveness following coronary stenting. Heparin as a possible aetiological factor in stent thrombosis. *Eur Heart J* 1998; **19**:1239–48.
17. Lau WC, Waskell LA, Watkins PB et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug–drug interaction. *Circulation* 2003;**107**:32–7.
18. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al. 807 C/T polymorphism of the glycoprotein Ia gene and pharmacogenetic modulation of platelet response to dual antiplatelet treatment. *Blood Coagul Fibrinol* 2004;**15**:427–33.
19. Fontana P, Dupont A, Gandrille S et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003;**108**:989–95.
20. Steinhubl SR, Talley JD, Braden GA et al. Point of care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention: results of the GOLD (AU-Assessing Ultegra) multicenter study. *Circulation* 2001;**103**:2528–30.
21. Kastrati A, Mehilli J, Schuhlen H et al. A clinical trial of abciximab in elective percutaneous coronary intervention after pretreatment with clopidogrel. *N Engl J Med* 2004;**350**:232–8.
22. Neumann FJ, Kastrati A, Pogatsa-Murray G et al. Evaluation of prolonged antithrombotic pre-treatment (''Cooling-off'' strategy) before intervention in patients with unstable coronary syndromes. *JAMA* 2003;**290**:1593–9.
23. Muller I, Seyfarth M, Rudiger S et al. Effect of high LD of clopidogrel on platelet function in patients undergoing coronary stent placement. *Heart* 2000;**85**:92–3.
24. Seyfarth HJ, Koksch M, Roethig G et al. Effect of 300- and 450-mg clopidogrel LDs on membrane soluble P-selectin in patients undergoing coronary stent implantation. *Am Heart J* 2002;**143**:118–23.
25. Quinn MJ, Bhatt DL, Zidar F et al. Effect of clopidogrel pretreatment on inflammatory marker expression in patients undergoing percutaneous intervention. *Am J Cardiol* 2004;**93**:679–84.
26. Steinhubl SR, Berger PB, Mann III JT et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial (CREDO). *JAMA* 2002;**288**: 2411–20.
27. Pache J, Pache J, Kastrati A et al. Clopidogrel therapy in patients undergoing coronary stenting:value of a high-LD regimen. *Cathet Cardiovasc Intervent* 2002;**55**:436–41.
28. Gum PA, Kottke-Marchant K, Welsh PA et al. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003;**41**:961–5.
29. Muller I, Besta F, Schulz C et al. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. *Circulation* 2003;**108**:2195–7.
30. Saw J, Steinhubl SR, Berger PB et al. Lack of adverse clopidogrel-atorvastatin clinical interaction from secondary analysis of a randomized, placebo-controlled clopidogrel trial. *Circulation* 2003; **108**:921–4.
31. Nikhil J, Reis M, Phillips AM et al. A randomized comparison of the effects of a high versus conventional loading dose of clopidogrel on platelet aggregation prior to percutaneous intervention. *Circulation* 2001;**104**(Suppl. II):347.

Downloaded from https://academic.oup.com/eurheartj/article/25/21/1903/455915 by guest on 21 August 2022