Statins do not adversely affect post-interventional residual platelet aggregation and outcomes in patients undergoing coronary stenting treated by dual antiplatelet therapy

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Received 4 July 2007; revised 17 April 2008; accepted 6 May 2008; online publish-ahead-of-print 24 May 2008

Aims

There are growing data suggesting a clinical relevance of residual platelet aggregation (RPA) in patients undergoing PCI. Drug–drug interaction of statins and clopidogrel has been controversially discussed in ex vivo studies and clinical trials. The aim of the present study was to investigate the effects of peri-procedural statin medication on the metabolism of aspirin and clopidogrel with regard to platelet aggregation and clinical outcome in patients undergoing coronary intervention.

Methods and results

Patients with coronary stenting for symptomatic coronary artery disease are routinely evaluated by platelet function analysis in a monocentre registry, and for the present study, a consecutive cohort of 1155 patients were analysed. About 87.7% of the patients were treated with statins at the time of platelet function analysis. Residual platelet activity assessed by adenosine diphosphate (20 μmol/L)-induced platelet aggregation was not significantly influenced by statin treatment. Nor the significant effects of CYP3A4-metabolization pathway on post-treatment aggregation were recorded, although there was even a trend to lower RPA values in patients treated with CYP3A4-metabolized statins. Further, in an inter-individual analysis comparing patients treated with CYP3A4- and non-CYP3A4-metabolized statins, no time-dependent difference of clopidogrel’s anti-aggregatory effects was observed. Clinical follow-up of major adverse events (myocardial infarction, ischaemic stroke, death) in 991 patients within 3 months revealed no significant adverse effects of statin treatment on clinical outcome. Instead, statin treatment was independently associated with lower incidence of composite events (HR 0.44, 95% confidence interval 0.23–0.83, P = 0.01).

Conclusion

Peri-procedural co-administration of statins does not increase the post-interventional RPA in cardiovascular patients treated with dual antiplatelet therapy and does not worsen the clinical prognosis of these patients.

Keywords

Stents • Interactions • Clopidogrel • Statins • CYP3A4 • Platelets

Introduction

Dual antiplatelet therapy (aspirin plus clopidogrel) after coronary stenting decreases the incidence of acute stent thrombosis and reduces the risk for myocardial infarction, stroke, and cardiovascular death.1,2 It has nowadays become the post-interventional anti-thrombotic standard of therapy.

Clopidogrel is an inactive prodrug that is administered orally and metabolized primarily in the liver. It has been demonstrated that the cytochrome P-450 isoform 3A4 (CYP3A4) is responsible for the hepatic activation of clopidogrel.3 Converted into its active compound, clopidogrel irreversibly inhibits the adenosine diphosphate (ADP)-induced platelet aggregation by forming a disulfide bond with the platelet P2Y12 (P2Y12) ADP receptor.4

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Recently, a high variability of platelet response to clopidogrel has been documented by several studies. In patients with ischaemic heart disease undergoing coronary stenting, clopidogrel is frequently administered in combination with HMG-CoA inhibitors (statins). Some statins (e.g. atorvastatin, simvastatin, lovastatin and cerivastatin) are metabolized predominantly by CYP3A4, whereas fluvastatin is metabolized by CYP2C9 and pravastatin mainly by CYP450 independent metabolic pathways. In an initial ex vivo platelet function study, it was suggested that atorvastatin, unlike other statins such as pravastatin, attenuates clopidogrel’s effectiveness in inhibiting platelet aggregation in a dose-dependent manner. A possible class effect of CYP3A4-metabolized statins interfering with clopidogrel by competitive inhibition of this cytochrome has been addressed in different studies. The investigations provided controversial results whether statins attenuate or do not influence the anti-aggregatory platelet efficacy of clopidogrel in terms of anti-aggregatory effects and outcome.

Recently, we and others demonstrated that inadequate inhibition of residual platelet activity after a 600 mg loading dose of clopidogrel is associated with increased risk for recurrent thrombo-ischaemic events after coronary stenting. Furthermore, clinical conditions that influence RPA have been described. The potential drug interactions between statins and clopidogrel could limit the effects of antplatelet treatment and have significant clinical impact in patients with coronary artery disease (CAD).

The aim of the present study was to consecutively evaluate the effect of statin co-administration with a 600 mg loading dose of clopidogrel followed by 75 mg maintenance dose on ex vivo platelet aggregation and clinical outcome in patients undergoing coronary stent implantation.

Methods

Study design and patient selection

Patients admitted to our clinic for coronary intervention of symptomatic CAD are routinely evaluated by platelet function analysis in a monocentre registry. The study was approved by the local Ethics Committee and signed informed consent was obtained from all patients. The enrolment period of the studied patients was March 2005 until December 2006. Medical treatment of the investigated patients including statin treatment was due to clinical practice. For the present analysis, we retrospectively evaluated a large consecutive unselected cohort of 1155 patients. Patients with known platelet function disorders and a treatment with GPIIb–IIIa inhibitors within 1 week prior to platelet function analysis were excluded. A loading dose of 600 mg clopidogrel was given to the majority of patients (85%) prior to PCI followed by a daily dose of 75 mg for at least 3 months. If patients were on a chronic clopidogrel treatment, additional loading dose of 300 mg was administered and platelet function was assessed earliest 24 h after loading. All patients received a standard dose of aspirin 100 mg daily before enrolment in the study, unless there were contraindications against aspirin use, e.g. risk of gastrointestinal bleeding or allergic reactions.

Blood sampling and platelet aggregation measurement

Patient blood was collected at least 6 h (median 24.8 h inter-quartile range 23.1 h) after first administration of 600 mg clopidogrel, when maximum platelet inhibition was achieved. A small number of patients (~15%) with chronic clopidogrel treatment received a loading dose of 300 mg clopidogrel before coronary intervention and were measured ≥24 h after loading. Blood samples were collected in 3.8% citrate plasma. Samples were centrifuged at 1000 r.p.m. for 10 min to obtain platelet-rich plasma (PRP) and additionally 10 min at 3500 r.p.m. to recover platelet-poor plasma (PPP). Platelet concentration of PRP was adjusted to 2 × 10^10 per μL by adding homologous PPP. Per cent platelet aggregation after stimulation with 20 μmol/L ADP was assessed by the turbidometric method using a Chronolog Lumi aggregometer with Aggro-Links Software.

Follow-up

Follow-up was intended for a time period of 3 months. Endpoints were the composite of myocardial infarction, ischaemic stroke, and death. The endpoints were assessed by reviewing patients’ charts on re-admission or telephone interview. Telephone interviewers were blinded on behalf of the results of platelet aggregation.

Statistics

The primary objective of this study was to evaluate the effect of peri-procedural statin treatment on RPA. Secondary/explorative objectives were to investigate the impact of CYP3A4-metabolized statin treatment and atorvastatin treatment on RPA and to assess the influence of metabolization pathway of different statins on clinical outcome as measured by composite events of death, myocardial infarction, and ischaemic stroke within the follow-up period. Categorical variables are presented as frequencies and percentages. We performed a χ² test to evaluate the distribution of categorical data and a Fisher’s exact test for dichotomous analysis. A Shapiro–Wilk test was performed to assess the normality of continuous variables. Normally distributed continuous data are expressed as mean ± standard deviation. For these variables, means between two categories were compared with a two-tailed unpaired t-test. Since hypothesis for normal distribution of RPA had to be rejected (Shapiro–Wilk), we preferred non-parametric statistical analysis to further test this parameter. The Wilcoxon–Mann–Whitney U test was performed to test for difference in RPA between two groups and the Kruskal–Wallis test was used for comparison of multiple groups. Rank-sum test was applied for statistical testing of RPA between time categories.

To adjust for possible confounders, a multivariable analysis was included. To allow for normality of residuals, we applied the Box–Cox transformation of RPA as a dependent variable (λ = 0.67). A multivariable linear regression analysis was then performed including statin co-medication, gender, age > 65 years, diabetes, tobacco use, arterial hypertension, hyperlipidaemia, ST-elevation myocardial infarction on admission, reduced left ventricular function (EF < 55%), renal failure, and cardiovascular medication [angiotensin-converting enzyme (ACE)-inhibitors, AT1 blockers, beta-blockers, diuretics] by the enter method in our model. The appropriateness of the regression model was judged from the Durbin–Watson statistic and partial plots of the residuals.

Time to first event analysis was calculated by the Kaplan–Meier method, and survival time across different groups was compared with the log-rank test. Multivariable Cox proportional hazards survival regression was used to investigate the effects of statin on outcome after adjustment for relevant factors influencing outcome (cardiovascular medication, left ventricular function, age, acute coronary syndromes, cardiovascular risk factors including diabetes, smoking, hypertension, hyperlipidaemia, and renal failure with a serum creatinine > 1.5 mg/dL). All analyses were two-sided and
P< 0.05 was considered statistically significant. The proportional hazard assumption was assessed by a plot of log(–log(survival function)) vs. time for combined major clinical endpoints. Statistical analysis was performed with SPSS® version 15 for windows (SPSS, Inc., Chicago, IL, USA) and Minitab®, version 15 for windows (Minitab Inc., State College, USA).

Results

One thousand four hundred and sixty-nine patients were initially assessed for inclusion into the study. Of these, 70 patients refused willing consent and 150 patients were deemed ineligible due to platelet function disorders or treatment with GPIIb–IIIa inhibitors within 1 week prior to possible enrolment. Ninety-four of the patients were initially included but remained without further measurements. For the present analysis, we investigated a consecutive cohort of 1155 patients. The mean (± SD) age of the patients in the total collective was 67 ± 11 years (range 30–94 years). Detailed characteristics of the patients are listed in Table 1. One thousand and thirteen patients (87.7%) received a peri-procedural statin treatment and 142 (12.3%) were not treated with statins. Among the patients with concomitant statin treatment, 25.9% refused willing consent and 150 patients were deemed ineligible.
received atorvastatin [mean dose (MD) 18.5 mg], 23.7% fluvastatin (MD 50.1 mg), 48.3% simvastatin (MD 24.6 mg), 1.7% pravastatin (MD 19.1 mg), and 0.4% lovastatin (MD 28.3 mg). In univariate analysis, there were significant differences between subgroups of patients with and without statin treatment regarding gender, age, tobacco use, co-administration of diuretics, ACE-inhibitors, and beta-blockers. The rate of patients with ST-elevation myocardial infarction and the proportion of women were higher in the CYP3A4 compared with the non-CYP3A4 subgroup (Table 1).

The median of residual ADP (20 μmol/L)-induced platelet aggregation of the tested 1155 patients was 36% (interquartile range 35.2). As shown in Figure 1, residual ADP-induced platelet aggregation was not statistically significantly different between subgroups of statin-treatment and patients without concomitant statin therapy. Especially, there was no difference between the group of CYP3A4-statins and non-CYP3A4-statins (Figure 2).

In multivariable linear regression analysis using platelet aggregation modified by the Box–Cox transformation as a dependent variable, statin treatment did not show a significant influence on RPA after adjustment for possible confounders [beta-coefficient –0.02, 95% confidence interval (CI): –0.95 to 0.91; P = 0.97]. In this model, reduced left ventricular function, older age, and renal failure had a significant influence on RPA. Beta-coefficients and their 95% CIs for all included variables are summarized in Table 2. The Durbin–Watson statistics was 1.94 indicating no significant autocorrelation in the residuals.

In a secondary explorative analysis, neither CYP3A4 statin nor atorvastatin treatment did show a significant influence on RPA (CYP3A4: beta-coefficient –0.2, 95% CI: –0.63 to 0.23, P = 0.36; atorvastatin: OR 0.01, 95% CI: –0.42 to 0.45, P = 0.95) after adjustment for the same variables.

In a time-dependent analysis of effects of inter-individual delay of measurement after first clopidogrel administration, we could not observe a significant difference between the CYP3A4 and non-CYP3A4 subgroups (Figure 3). Follow-up was intended for a time period of 3 months. For 991 patients (85.8%), follow-up data were available either by telephone interview or by clinical data recordings. Three-month follow-up could be completed in 776 patients (78.3%). Fifty-seven patients had a composite event within 90 days (5.8% of the followed-up patients), 23 patients died (2.3%), 26 patients suffered from myocardial infarction (2.6%), and eight patients developed an ischaemic stroke (0.8%). Kaplan–Meier analysis of major adverse events revealed no significant difference of event-free survival in patients with statin or without statin treatment (log rank P < 0.001; Figure 4). There was no significant difference in survival analysis regarding the subgroups of CYP3A4 and non-CYP3A4 statin treatment (log rank P = 0.63; Figure 5A) nor in patients receiving atorvastatin compared with patients treated with other statins (log rank P = 0.45, Figure 5B). After adjustment for age, gender, cardiovascular risk factors, and relevant cardiovascular medication, statin co-administration was independently associated with a significant better outcome [hazard ratio (HR) 0.44, 95% CI: 0.23–0.83, P = 0.01; gender HR 0.76, 95% CI: 0.41–1.41, P = 0.38; arterial hypertension HR 0.82, 95% CI: 0.42–1.6, P = 0.55; diabetes HR 2.24, 95% CI: 1.27–3.97, P = 0.006; hyperlipidaemia HR 0.66, 95% CI: 0.37–1.18, P = 0.16; tobacco use HR 0.6, 95% CI: 0.3–1.21, P = 0.15; acute coronary syndromes HR 2.23, 95% CI: 1.19–4.18, P = 0.01; age >65 years HR 2.09, 95% CI: 0.98–4.47, P = 0.06; reduced left-ventricular function HR 2.81, 95% CI: 1.44–5.48, P = 0.01; diabetes HR 0.6, 95% CI: 0.3–1.21, P = 0.15].

Figure 1 Comparison of post-treatment platelet aggregation between patients receiving peri-inventional statin treatment and patients without statin treatment (primary objective) and subgroups of statin treatment (secondary objective). Each box shows the median of platelet aggregation including quartiles and the extreme values that fall within 1.5 inter-quartile range (whiskers) for each subgroup.

Figure 2 Comparison of post-treatment platelet aggregation between different subgroups of CYP3A4 and non-CYP3A4-metabolized statins and patients treated with atorvastatin and statins other than atorvastatin (secondary objectives). Each box shows the median of platelet aggregation including quartiles and the extreme values that fall within 1.5 inter-quartile range (whiskers) for each subgroup.
The results of the present study suggest that pre-administration of different types of statins before a 600 mg clopidogrel loading does not significantly influence the residual platelet activity and clinical outcome.

**Discussion**

The results of the present study suggest that pre-administration of different types of statins before a 600 mg clopidogrel loading does not significantly influence the residual platelet activity and clinical outcome.
outcome in patients with symptomatic CAD treated by coronary stenting. To our knowledge, this is the largest study investigating the influence of statins both on ex vivo platelet aggregation and clinical outcome in an unselected, consecutive cohort of patients with symptomatic CAD.

Prior studies suggested a possible attenuation of antiplatelet effects of clopidogrel by lipophilic statins, particularly atorvastatin via competitive inhibition of hepatic CYP3A4. However, the present data contribute to the growing body of evidence that these observations are of no clinical relevance in cardiovascular patients treated with dual antiplatelet therapy and a standard statin dosing regimen.

Lipophilic statins, such as atorvastatin, are substrates of CYP3A4, the enzyme responsible for conversion of clopidogrel to its active metabolite. It is suggested that CYP3A4 statins decrease the antiplatelet compounds of clopidogrel by competitive inhibition. This effect might be amplified in patients with genetic polymorphism affecting the cytochrome P450 activity like functional variants of CYP3A4, CYP3A5, or CYP2C19.

With the present results, we demonstrate that regardless of the nature of statin substance, platelet inhibition by clopidogrel was not modified. Instead, we even observed a trend towards lower RPA in patients treated with CYP3A4-metabolized statins (Figure 2). Moreover, there was a trend for better survival in the same group (Figure 5A). It cannot be ruled out that the CYP3A4–clopidogrel interaction is a dose-dependent phenomenon, as it is known that the degree of competitive inhibition between two substrates depends on their effective concentrations.

Nevertheless, with conventional dosing regimen, we did not observe a dose-dependent correlation with RPA.

Although investigated by inter-individual measurements, our results suggest that there are no effects of different statin treatments on metabolization of clopidogrel as we did not observe time-dependent effects of platelet inhibition after 6 h when maximum effects are expected. An attenuating effect of CYP3A4 substrates on clopidogrel metabolism has been previously discussed. However, this observation could not be confirmed by kinetic studies on platelet function early (up to 4 h) after a 600 mg clopidogrel loading dose and statin administration.

In a follow-up period of 3 months, we did not observe a difference on behalf of major adverse events (death, myocardial infarction, ischemic stroke) between subgroups of statin treatment. Thus, the data are consistent with the previous subgroup analysis from the CREDO (n = 1159 patients, 300 mg loading dose + statin treatment) and MITRA-PLUS trials (n = 2086 patients, clopidogrel standard dosing + statin treatment at discharge), which demonstrated no adverse effects of clopidogrel–statin co-administration on clinical endpoints after 28 days, 1 year, and 14 months, respectively. There was a significant unexpectedly high rate of events in patients who were not treated with statins at the time of enrolment (17.2 vs. 6.4 within follow-up). These results might have been influenced by the fact that patients without statin therapy were significantly older, had more often renal failure, and
were less often treated with beta-blockers and ACE-inhibitors and more often treated with diuretics. Although we did not systematically evaluate the continuation of statin therapy and further changes in dose, we found that the majority (around 83%) of the patients who were not initially treated with statins did not receive a statin at follow-up time. Thus, the observed worse outcome in the group of patients with no initial statin treatment might partly be due to the missing long-term prognostic benefits. To account for possible confounding variables, we performed a multivariable Cox regression analysis including significant factors in univariate analysis and other relevant factors that influence the outcome and found that besides acute coronary syndromes, diabetes, and reduced left ventricular function, co-medication with statins was independently associated with lower incidence of events (HR 0.44, 95% CI 0.23–0.83, \( P = 0.01 \)). To our opinion, the lower incidence of events in the statin-treated group might be partly due to positive drug effects.

We are aware that the results presented imply several limitations. First, this is a non-randomized, single-centre study and we only investigated one single platelet function marker. Furthermore, the interaction of statins and clopidogrel might be influenced by the long-term administration. We were not able to assess the long-term administration of statins and neither did we systematically evaluate further dosage of particular statins. However, we found that peri-procedural administration of statins independently of metabolization pathway does not affect the RPA measured around 24 h after a 600 mg loading dose and does neither affect the short-term outcome. In the recently published subgroup analysis of the CHARISMA trial, Saw et al.\(^{11}\) found no long-term adverse effects of CYP3A4-metabolized statin treatment on the outcome of patients with multiple cardiovascular risk factors receiving chronic clopidogrel treatment (median follow-up: 28 months).

Besides, we did not further specify events regarding the occurrence of stent thrombosis. The inefficacy of clopidogrel-dependent platelet inhibition has been previously described to correlate particularly with the occurrence of subacute stent thrombosis.\(^{30–32}\) Most recently, Wenaweser et al.\(^{33}\) showed in a small randomized study of patients with previous stent thrombosis that there was

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**Figure 5** Kaplan–Meier analysis of a combined major adverse event (death, myocardial infarction, ischaemic stroke) in 991 patients dependent on subgroups of patients initially treated with a CYP3A4-metabolized or a non-CYP3A4-metabolized statin (A) and patients treated with atorvastatin or with other statins than atorvastatin (B).
The work was supported in part by the Deutsche Forschungsgemeinschaft, the Wilhelm Sander Stiftung and the Fortune-Program of the University of Tübingen.

Funding

The work was supported in part by the Deutsche Forschungsgemeinschaft, the Wilhelm Sander Stiftung and the Fortune-Program of the University of Tübingen.

Conflict of interest: none to declare.

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