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Impact of cytochrome P450 3A4-metabolized statins on the antiplatelet effect of a 600-mg loading dose clopidogrel and on clinical outcome in patients undergoing elective coronary stent placement

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Summary

Early studies suggested interactions between statins and clopidogrel. Based on the outcome and platelet data, there is now huge evidence of no interactions between statins and 75 to 300 mg clopidogrel; however, data with 600-mg loading are lacking. In a pre-specified analysis of the EXCELSIOR cohort, we investigated the interaction between statins, especially cytochrome P4503A4-metabolized atorvastatin and simvastatin, and the antiplatelet effects of a 600-mg loading dose of clopidogrel. We analyzed 1,395 patients scheduled for coronary angiography (CA). Patients received clopidogrel 600 mg at least two hours before CA and 75 mg daily thereafter in case of percutaneous coronary intervention (PCI). Statin medication on admission was continued unaltered until discharge. Platelet function was assessed by optical aggregometry and flow cytometry of adenosine diphosphate (ADP)-stimulated surface expression of CD62P, CD63 and PAC-1 before clopidogrel and immediately before CA. Residual platelet aggregation (RPA) after addition of ADP 5 µM was similar irrespective of statin treatment at baseline (p=0.968). RPA at CA was 46.2 ± 16.8% in patients without statin (n=682), 45.5 ± 17.0% in patients with atorvastatin (n=255), 45.8 ± 16.3% with simvastatin (n=335), 47.3 ± 14.9% with fluvastatin (n=42) and 45.9 ± 16.2% with pravastatin (n=81; p=0.962). Consistent results were obtained by flow cytometry. In patients with PCI (n=553), the one-year incidence of death, myocardial infarction and target lesion reintervention did not differ between cohorts stratified according to statin co-medication (p=0.645). Thus, peri-interventional atorvastatin and simvastatin had no effect on the antiplatelet activity of a loading dose of clopidogrel 600 mg and did not affect clinical outcome after PCI.

Introduction

The thienopyridine derivative clopidogrel is an inactive pro-drug which is converted by cytochrome P450 3A4 (CYP3A4) into the active metabolite (1, 2). This active metabolite binds irreversibly to the platelet purinergic P2Y$_{12}$-receptor and thereby inhibits adenosine diphosphate (ADP)-induced platelet activation (2). Previous studies suggested that the HMG-CoA-reductase inhibitor atorvastatin might attenuate the platelet inhibitory effect of clopidogrel probably by a metabolic drug-drug interaction via interference at the level of cytochrome P450 3A4 (3, 4). Subsequent studies investigating the interaction of CYP3A4-metabolized statins with the antiplatelet effects of clopidogrel failed to confirm these findings (5–10). The statistical power of the latter investigations, however, is limited due to low sample sizes.

Concerning clinical outcome data, analysis of three randomized trials (11–13) and one registry (14) did not provide evidence for an unfavourable outcome of patients on clopidogrel with concomitant administration of CYP3A4-metabolized statins. Contradictory to these findings, a pharmaco-epidemiological study showed an association between the concomitant use of clopidogrel and atorvastatin and adverse cardiovascular events within 30 days following percutaneous coronary intervention (PCI) (15).

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Thus, in the absence of prospective data on antiplatelet efficacy of clopidogrel in appropriately sized cohorts, the relevance of the interaction between clopidogrel and statins especially CYP3A4-metabolized statins (atorvastatin, simvastatin) is still unclear. In a pre-specified secondary analysis of the EXCELSIOR cohort (16, 17), we therefore investigated the impact of different statin regimens on the antiplatelet effects of a 600-mg loading dose of clopidogrel measured *ex vivo* at the time of PCI. We expanded our preliminary investigation (16) by a substantial increase in the number of patients enrolled and by restricting the analysis to patients with cardiac catheterization performed not earlier than two hours after administration of the 600-mg loading dose of clopidogrel. Moreover, we assessed the 12-month clinical event rate in a sub-cohort of patients with coronary stent placement.

Subjects and methods

Study population
The study was designed as a pre-specified secondary analysis of the EXCELSIOR cohort (16, 17). Details of the study design have been published previously (17). Patients with symptomatic coronary heart disease scheduled for cardiac catheterization as potential candidates for a PCI were eligible for the study. For the current analysis, we selected patients in whom statin medication had not been altered within the 14 days before admission or who were not on statin treatment for the last 14 days. Statin medication on admission was not changed until discharge. All patients were on chronic treatment with aspirin ($\geq$ 100 mg per day). We did not include patients with acute myocardial infarction (MI) according to the European Society of Cardiology/American College of Cardiology (ESC/ACC) criteria (18), with thienopyridine treatment within the last 14 days before admission, with chronic oral anticoagulation, with contraindications to either aspirin, clopidogrel or heparin as well as patients with bleeding diathesis, malignancies or hematological disorders including thrombocytopenia ($< 100x10^9$/l). In the current analysis we also excluded patients who underwent cardiac catherization within two hours after clopidogrel loading. Glycoprotein IIb/IIIa inhibitors were not allowed except for bail-out. The use of drug-eluting stents was permitted.

The study protocol was approved by the ethics committee of the medical faculty of the University of Freiburg, Germany, and complies with the Declaration of Helsinki. All patients provided written informed consent.

Study protocol and blood sampling
Immediately after inclusion in the study, blood was drawn for baseline platelet function assays using tubes containing 3.8% sodium-citrate (Sarstedt AG, Nuembrecht, Germany). Thereafter, an oral dose of clopidogrel 600 mg was administered. The second blood sample was obtained during catheterization before administration of heparin or contrast medium. Catheterization of the study patients was performed according to the routine schedule of the catheterization laboratory. In patients with coronary stent placement, a further blood sample for platelet function assays was obtained in the morning of the day after PCI between 2–4 hours after ingestion of the first maintenance dose of clopidogrel 75 mg. In these patients, continuation of treatment with clopidogrel 75 mg/day was recommended for four weeks in those receiving a bare metal stent and for six months in those receiving a drug-eluting stent.

Platelet aggregometry
Platelet aggregation was assessed by light transmission aggregometry using a four-channel Bio/Data PAP4 aggregometer (Mölab, Langenfeld, Germany), as described previously (16). Platelet-rich plasma (PRP) was prepared by centrifugation of citrated venous blood at 750g for 2 minutes (min) and adjustment to $275–325x10^9$ thrombocytes/l by dilution with platelet-poor plasma (PPP) from the same patient. Residual platelet aggregation (RPA) was determined as light transmission in platelet-rich plasma 5 min after addition of ADP (Sigma, Munich, Germany) at final concentrations of 5 as well as 20 μM. Percentage of maximal light transmission was calculated using PPP from the same patient as reference (= 100% aggregation). We focussed on RPA because RPA most closely reflects the clopidogrel-dependent inhibition of $P2Y_{12}$ purinergic receptors (19), whereas peak aggregation, used in other studies (20–23), is influenced by both, $P2Y_1$ and $P2Y_{12}$. The coefficient of variation of our optical aggregometry assay is 6.1% (16).

RPA after stimulation with 5 μM ADP served as the primary measure for assessment of antiplatelet effect of clopidogrel, because it proved to be highly predictive for major adverse coronary events (MACE) within 30 days after stent placement in EXCELSIOR (17).

Whole blood flow cytometry
Platelet surface expression of P-selectin (CD62P-PE, Beckman-Coulter, Krefeld, Germany), CD63 (CD63-FITC, Beckman-Coulter, Krefeld, Germany) and activated GPIIb/IIIa (PAC-1-FITC, Becton Dickinson, Heidelberg, Germany) was determined by triple color flow cytometry as described previously (16). Platelets were identified in whole blood by size and a platelet-specific monoclonal antibody (CD41-PC7, Coulter, Krefeld, Germany) after incubation with ADP 20 μM and antibodies for 30 min. Thereafter, 300 μl of para-formaldehyde 1% was added for fixation. A four-channel flow cytometer equipped with a 488 nm argon laser (FACSCalibur, Becton Dickinson, Heidelberg, Germany) was used, and 10,000 events from each sample were analyzed. The mean channel of fluorescence intensity was taken as a measure for antibody binding and thus antigen surface exposure.

Clinical follow-up
Patients undergoing PCI were monitored by systematic serial assessment of creatinine kinase and troponin T as well as ECG recordings until discharge. Follow-up information at 12 months after PCI was obtained in all patients with PCI (n=553). MACE including death from any cause, MI and target lesion reintervention (TLR) from 24 hours after PCI up to 12 months were recorded, thus excluding acute procedure-related events. The diagnosis of MI was made according to the ESC/ACC consensus document and based on new rise in troponin T $\geq$0.03 mg/l associated with either typical symptoms and/or typical electrocardiographic changes and/or typical angiographic findings (16, 18).
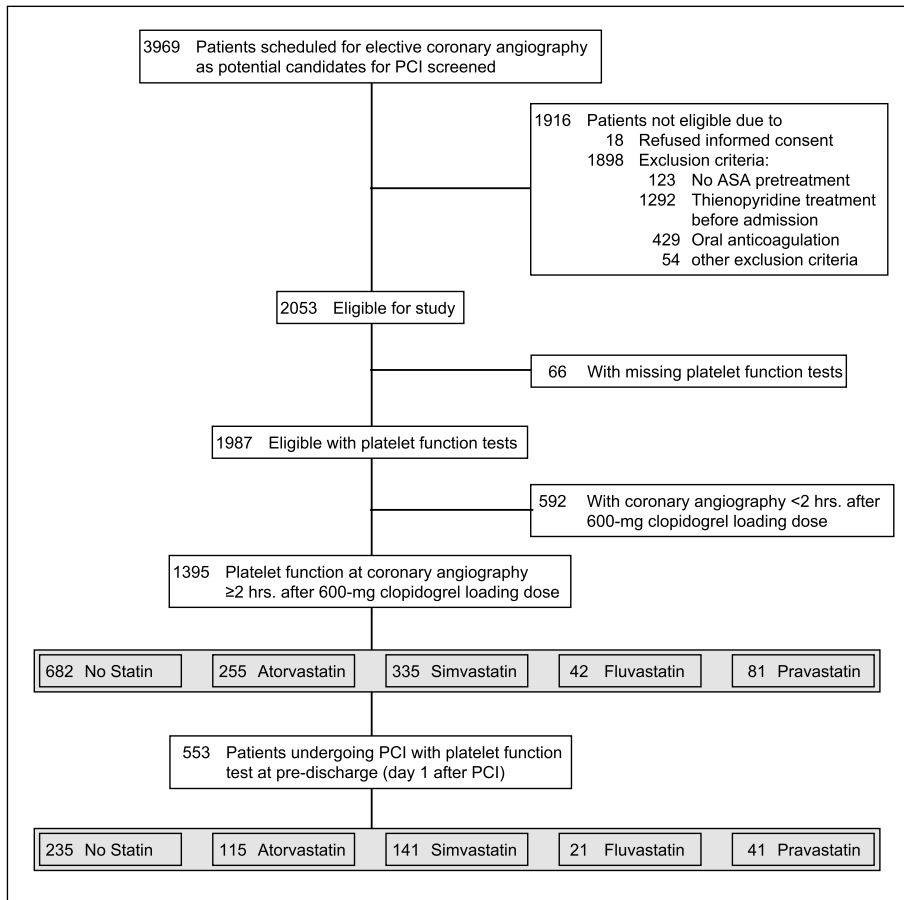
Figure 1: Trial profile.

All events were classified and adjudicated by two physicians not involved in the follow-up process.

Statistics

Discrete variables are reported as counts (percentages) and continuous variables as mean ± standard deviation. Differences between groups were analyzed with the Chi^2-test for discrete variables and with one-way ANOVA for continuous variables. One-way ANOVA was also used for assessment of dose-related effects of statins.

Our analyses were based on five predefined groups: patients without treatment with statin, patients on CYP3A4-metabolized statins (atorvastatin, simvastatin), and patients with statins not metabolized by CYP3A4 (fluvastatin, pravastatin). We primarily tested the hypothesis that platelet aggregation at the time of catheterization differed between these five groups. To correct for potential bias by differences in baseline demographic and clinical variables we used the general linear model. This model included all variables which showed a significant difference (p <0.05) between the five treatment groups. We assessed the difference in the incidence of clinical endpoints between the strata defined by statin treatment by two-sided testing based on the log-rank statistic. Cumulative event rates were calculated and graphically described according to the Kaplan-Meier method. SPSS software package, version 14.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. In the two-tailed test, a p-value <0.05 was regarded as significant.

With the given sample size and a common standard deviation of 18% for RPA our study had an 80% power to detect a 3% difference in residual platelet aggregation at a p <0.05 (nQuery for Windows, version 5.0; Statistical Solutions, Cork, Ireland).

Results

Patient characteristics

Figure 1 shows the study flow-chart. We enrolled 1,395 consecutive patients from the initial cohort of 1,987 patients and excluded 592 patients with cardiac catheterization <2 hours after administration of the 600-mg bolus dose of clopidogrel. Table 1 shows the baseline demographic and clinical characteristics of the study cohort. Patients without statin medication on admission differed significantly from patients treated with statins. Patients with lipid-lowering therapy more often had a history of previous MI, previous PCI or coronary artery bypass grafting (CABG). Definite treatment after the index angiography was more frequently conservative therapy in patients with no current statin therapy.

Except for a more frequent history of previous MI and lower cholesterol levels in patients treated with atorvastatin and simvastatin, there were no significant differences between the patient groups defined by prescription of various statins.

Table 1: Baseline demographic and clinical characteristics of the study cohorts.

	No Statin (n = 682)	Atorvastatin (n = 255)	Simvastatin (n = 335)	Fluvastatin (n = 42)	Pravastatin (n = 81)	P*	P†
Dose of statin (mg)		19.8 ± 9.2	23.7 ± 12.6	54.3 ± 24.7	27.9 ± 13.7	-	-
Time to catheterization (hours)#	7.0 ± 10.7	8.2 ± 8.6	8.4 ± 12.9	10.6 ± 11.9	8.0 ± 10.7	0.106	0.599
Age (years)	64.9 ± 9.8	65.9 ± 8.3	66.0 ± 9.6	64.6 ± 11.6	66.4 ± 10.2	0.324	0.778
Women	278 (40.8)	65 (25.5)	89 (26.5)	12 (28.6)	23 (28.4)	<0.001	0.945
Hypercholesterolemia	539 (78.9)	244 (95.7)	314 (93.7)	40 (95.2)	80 (98.8)	<0.001	0.271
Active smoker	87 (12.7)	20 (7.8)	37 (11.0)	9 (21.4)	7 (8.6)	0.056	0.068
Arterial hypertension	503 (73.8)	207 (81.2)	277 (82.4)	28 (66.7)	68 (84.0)	0.002	0.080
Diabetes mellitus	126 (18.4)	62 (24.3)	84 (25.0)	10 (23.8)	17 (21.0)	0.108	0.900
Body mass index (kg/m²)	27.7 ± 4.0	28.3 ± 4.1	27.9 ± 4.0	28.0 ± 3.6	28.3 ± 3.2	0.215	0.736
Previous balloon angioplasty	88 (12.9)	137 (53.7)	168 (50.0)	16 (38.1)	32 (39.5)	<0.001	0.062
Previous CABG	42 (6.1)	47 (18.4)	49 (14.6)	11 (26.2)	14 (17.3)	<0.001	0.239
Previous myocardial infarction	77 (11.3)	93 (36.5)	100 (29.8)	10 (23.8)	14 (17.3)	<0.001	0.007
Reduced left ventricular function	174 (25.5)	100 (39.2)	127 (37.8)	17 (40.5)	25 (30.9)	<0.001	0.575
Beta blockers	373 (54.7)	197 (77.3)	269 (80.1)	34 (81.0)	56 (69.1)	<0.001	0.169
ACE inhibitors	240 (35.2)	138 (54.1)	170 (50.6)	18 (42.9)	40 (49.4)	<0.001	0.538
Diuretics	199 (29.2)	90 (35.3)	120 (35.7)	11 (26.2)	33 (40.7)	0.054	0.462
Nitrates	134 (19.6)	93 (36.5)	99 (29.5)	12 (28.6)	21 (25.9)	<0.001	0.184
Oral antidiabetics	69 (10.1)	36 (14.1)	44 (13.1)	6 (14.3)	11 (13.6)	0.380	0.987
Definite treatment							
PCI	235 (34.4)	115 (45.1)	141 (42.0)	21 (50.0)	41 (50.6)	0.001	0.467
CABG	33 (4.8)	5 (2.0)	14 (4.2)	2 (4.8)	2 (2.4)	0.300	0.364
Medical therapy	413 (60.6)	135 (52.9)	181 (53.8)	19 (45.2)	38 (47.0)	0.018	0.527

Data are expressed as mean value ± SD or number of patients (percentage). P*, chi²-test or Fisher's exact test for categorical variables and one-way ANOVA for continuous variables between all strata. P†, chi²-test or Fisher's exact test for categorical variables and one-way ANOVA for continuous variables betweens strata of patients treated with statins. #, time from administration of clopidogrel 600 mg to cardiac catheterization; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting.

Residual platelet aggregation (RPA)

RPA after stimulation with 5 µM ADP determined at baseline before administration of clopidogrel and at the time of coronary catheterization is shown in Table 2. In all groups defined by statin medication RPA had decreased in the sample drawn immediately before coronary angiography as compared with baseline. We did not find any significant differences in RPA between the five cohorts, either at baseline before administration of clopidogrel (p = 0.968) or at the time of cardiac catheterization (p = 0.962).

The mean difference in RPA (5 µM ADP) between patients without statin medication and patients with CYP3A4-statin treatment without adjustment for differences in baseline characteristics was –0.81% (95% confidence interval [CI]: –3.86% to 2.24%; p=0.604). Adjustment for pertinent demographic and clinical variables (gender, hypercholesterolemia, arterial hypertension, previous PCI, CABG or MI, medical treatment with a β-blocker, an ACE-inhibitor or a nitrate, stent implantation) resulted in a mean difference in RPA between patients without statins and patients with CYP3A4-statin treatment of –1.25% (95% CI: –3.24% to 0.74%; p = 0.217). Differences in RPA between patients with CYP3A4-statins and non-CYP3A4-statins were 1.16% (-1.93% to 4.25%; p = 0.460) for unadjusted and 0.36% (-2.72% to 3.44%; p = 0.817) for adjusted analyses.

The daily doses of atorvastatin and simvastatin ranged from 10 to 40 mg, with an average of 19.8 ± 9.2 mg for atorvastatin and

Table 2: Residual platelet aggregation following stimulation with ADP 5 or 20 μM and inhibition of surface protein expression at time of PCI and effect of concomitant statin therapy.

	No Statin (n = 682)	Atorvastatin (n = 255)	Simvastatin (n = 335)	Fluvastatin (n = 42)	Pravastatin (n = 81)	P*
Residual platelet aggregation 5 μM ADP (%)						
Baseline	46.2 ± 16.8	45.5 ± 17.0	45.8 ± 16.3	47.3 ± 14.9	45.9 ± 16.2	0.968
Coronary angiography	14.8 ± 16.1	15.2 ± 15.2	15.2 ± 16.2	14.4 ± 15.0	13.9 ± 15.5	0.962
Residual platelet aggregation 20 μM ADP (%)						
Baseline	73.4 ± 11.9	72.8 ± 11.9	73.3 ± 11.7	75.0 ± 10.5	73.4 ± 12.7	0.845
Coronary angiography	31.1 ± 23.9	33.0 ± 22.4	32.7 ± 24.1	34.8 ± 23.3	30.0 ± 23.3	0.579
Inhibition of surface protein expression (%)						
P-selectin	67.6 ± 29.2	65.7 ± 33.8	67.1 ± 24.1	67.8 ± 33.9	71.2 ± 24.3	0.728
Activated GP IIb/IIIa (PAC-1)	32.2 ± 31.3	32.8 ± 25.5	30.0 ± 26.3	37.1 ± 28.8	39.2 ± 25.5	0.131
CD63	31.1 ± 24.5	33.5 ± 16.3	32.1 ± 19.7	34.4 ± 19.3	33.9 ± 17.1	0.519

Inhibition of surface protein expression in % relative to baseline. Data are expressed as mean value ± SD. P*, one-way ANOVA between groups.

23.7 ± 12.6 mg for simvastatin. As shown in Table 3, there were no significant differences in RPA after loading with clopidogrel depending on the daily dose of either atorvastatin or simvastatin (p = 0.894 and p = 0.767, respectively).

Confirming the results obtained after stimulation with 5 μM ADP, RPA after stimulation with 20 μM ADP showed no significant differences between the groups stratified according to statin co-medication (Table 2).

Effect of statin treatment on the inhibition of ADP-stimulated surface expression of platelet receptors by clopidogrel

Inhibition of ADP-stimulated surface expression of platelet receptors after loading with clopidogrel was similar irrespective of treatment with atorvastatin, simvastatin, fluvastatin or pravastatin or no statin medication (Table 2). No dose-related differences regarding the inhibition of expression of surface proteins P-selectin, CD63 or PAC-1 were observed in patients treated with atorvastatin or simvastatin (Table 3).

Platelet function assays and clinical outcome in patients undergoing PCI

We obtained consistent results in the subgroup of patients undergoing PCI (n=553) in whom an additional assessment of platelet function could be performed at pre-discharge on the day following PCI. Treatment with a statin did not affect RPA after stimulation with ADP 5 or 20 μM, or the inhibition of the expression of surface proteins at any time after loading with clopidogrel (data not shown).

The 12-month composite rate of death and MI was 3.6% (20/553 patients) among the patients undergoing PCI (Table 4). There were no differences in the incidence of these adverse events between patients undergoing PCI without and with statin medication (Table 4). Likewise, there were no significant differences in the cumulative one-year incidence of death and myocardial infarction between strata defined by various statins or no statin (log-rank p = 0.558) as shown in Figure 2. Inclusion of tar-

get lesion revascularization increased the composite rate of post-procedural events to 12.7% (70/553). Again, the incidence of events did not differ according to statin treatment (Table 4).

Discussion

As the main result of our pre-specified secondary analysis of the EXCELSIOR cohort, we found that the CYP3A4-metabolized statins atorvastatin and simvastatin, at dosages used in daily practice do not attenuate the antiplatelet effect of a 600-mg loading dose of clopidogrel. With 1,395 patients included, our study was sufficiently powered to detect even minor differences in the effect of clopidogrel depending on statin treatment and, to the best of our knowledge, constitutes the largest cohort investigated for this issue. Consistent with the platelet function data, analysis of the sub-group of patients undergoing PCI did not suggest that concomitant treatment with CYP3A4-metabolized statins exposes the patients to an increased risk of thrombotic adverse events after stent placement.

Thus, our study clarifies the conflict between previously published results obtained in much smaller cohorts of patients scheduled for elective PCI (6–10) or patients with acute coronary syndromes (5, 12) and previous mechanistic studies (1, 4). Contrary to Lau et al. (4), we did not find any relationship between the dose of atorvastatin and RPA, with consistent results for simvastatin. Lau et al. (4) described an attenuated antiplatelet efficacy of a loading dose of clopidogrel 300 mg in a group of 19 patients treated with atorvastatin. Because the antiplatelet effects of the same loading-dose regimen of clopidogrel were preserved in patients under concurrent treatment with the non-CYP3A4 substrate pravastatin, it was suggested that inhibition of the metabolic activation of clopidogrel via CYP3A4 by atorvastatin was responsible for the blunted antiplatelet effects of clopidogrel in the patients treated with atorvastatin (4). The major contribution of CYP3A4 to the conversion of clopidogrel into the platelet-inhibiting metabolite was supported by additional experiments with inducers and inhibitors of CYP3A4.

Table 3: Residual platelet aggregation following stimulation with ADP 5 or 20 μM and inhibition of surface protein expression at time of PCI and dose-related effect of concomitant treatment with atorvastatin or simvastatin.

	10 mg	20 mg	40 mg	P*
Residual platelet aggregation 5 μM ADP (%)				
Atorvastatin	n = 74	n = 146	n = 35	
Baseline	48.6 ± 16.1	45.3 ± 18.0	41.7 ± 13.3	0.130
At coronary angiography	15.4 ± 16.3	15.4 ± 15.4	14.1 ± 12.4	0.894
Simvastatin	n = 69	n = 179	n = 87	
Baseline	46.4 ± 13.6	45.2 ± 16.9	46.6 ± 17.2	0.770
At coronary angiography	16.4 ± 18.5	14.8 ± 15.6	15.1 ± 15.4	0.767
Residual platelet aggregation 20 μM ADP (%)				
Atorvastatin	n = 74	n = 146	n = 35	
Baseline	73.5 ± 12.4	72.5 ± 11.9	72.2 ± 10.8	0.800
At coronary angiography	31.8 ± 22.8	33.4 ± 22.7	33.9 ± 21.1	0.857
Simvastatin	n = 69	n = 179	n = 87	
Baseline	75.2 ± 10.8	72.5 ± 11.0	75.0 ± 11.5	0.109
At coronary angiography	35.3 ± 25.6	31.4 ± 23.4	33.5 ± 24.3	0.504
Inhibition of surface protein expression (%)				
Atorvastatin	n = 74	n = 146	n = 35	
P-selectin	64.6 ± 35.3	65.7 ± 34.1	68.0 ± 29.5	0.904
Activated GP IIb/IIIa (PAC-1)	31.8 ± 28.5	33.0 ± 23.0	34.2 ± 29.6	0.901
CD63	34.4 ± 18.8	32.4 ± 15.1	36.5 ± 14.9	0.412
Simvastatin	n = 69	n = 179	n = 87	
P-selectin	68.0 ± 19.6	66.1 ± 23.4	68.1 ± 28.6	0.781
Activated GP IIb/IIIa (PAC-1)	27.1 ± 21.8	29.6 ± 27.7	33.3 ± 26.6	0.360
CD63	32.8 ± 13.6	32.5 ± 15.8	31.0 ± 28.7	0.821

Inhibition of surface protein expression in % relative to baseline. Data are expressed as mean value ± SD. P*, one-way ANOVA between groups.

Table 4: Major adverse cardiac events from 24 hours after PCI up to 12 months after PCI.

	No Statin (n = 235)	Atorvastatin (n = 115)	Simvastatin (n = 141)	Fluvastatin (n = 21)	Pravastatin (n = 41)	P*
Death or MI (total)	7 (3.0)	4 (3.5)	6 (4.3)	0 (0.0)	3 (7.3)	0.596
Death	2 (0.9)	1 (0.9)	3 (2.1)	0 (0.0)	2 (4.9)	0.268
Q-wave MI	0 (0.0)	1 (0.9)	1 (0.7)	0 (0.0)	0 (0.0)	0.426
Non Q-wave MI	5 (2.1)	2 (1.7)	2 (1.4)	0 (0.0)	1 (2.4)	0.952
TLR	22 (9.4)	11 (9.6)	17 (12.1)	2 (9.5)	2 (4.9)	0.766
Any MACE	27 (11.5)	13 (11.3)	23 (16.3)	3 (14.3)	4 (9.8)	0.645

Data are expressed as number of patients (percentage). P*, Chi² between groups. MI, non-fatal myocardial infarction defined according to the European Society of Cardiology/American College of Cardiology consensus document and based on new rise in troponin T ≥0.03 mg/l associated with either typical symptoms and/or typical ECG changes and/or typical angiographic findings; TLR, target lesion reintervention; MACE, major adverse cardiac events.

Several reasons may account for the conflicting results between the original observation by Lau et al. (4) and the results of the present study. It has to be considered that different methods were used for the assessment of antiplatelet effects of clopidogrel. Lau et al. (4) used a method based on platelet counting (ICHOR cell counter), while all other studies in this field used either optical platelet aggregometry (5–9), flow-cytometry of the expression of specific activation-dependent platelet surface proteins (5, 7–10) or a wide selection of platelet function assays including two cartridge based platelet analyzers (9). Furthermore, it has to be considered that the 300-mg loading dose of clopidogrel used by Lau may be more sensitive to the interaction with CYP3A4-metabolized statins than the 600-mg loading dose used in the current study (24). Because atorvastatin/simvastatin and clopidogrel are competitive substrates for the CYP3A4 enzyme system, the degree of competitive inhibition depends on
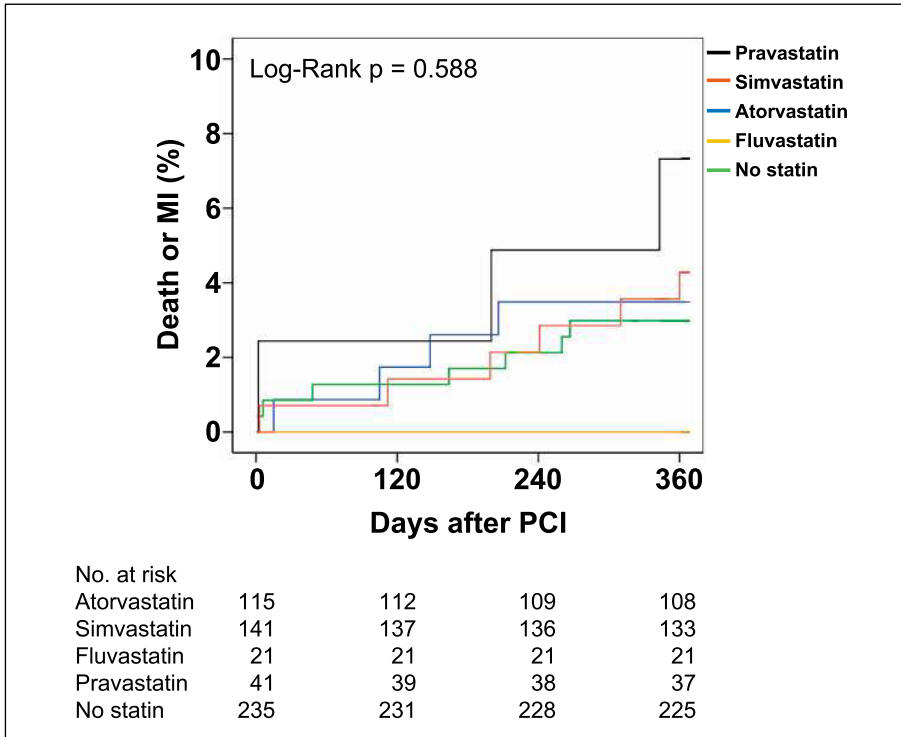
No. at risk

Atorvastatin	115	112	109	108
Simvastatin	141	137	136	133
Fluvastatin	21	21	21	21
Pravastatin	41	39	38	37
No statin	235	231	228	225

Figure 2: Cumulative composite incidence of death and myocardial infarction (MI) during 12-month follow-up. Cumulative composite incidence of death and MI from pre-discharge to one-year stratified by statin medication at PCI.

their relative affinities for the binding site and their relative concentrations. In the Interaction Study, however, a variety of platelet function assays did not demonstrate an attenuation of the antiplatelet activity of clopidogrel by various statins after the 300-mg loading dose of clopidogrel (9). Also, the antiplatelet effects of clopidogrel assessed after a treatment period of five weeks using a dosing scheme of 75 mg daily were not affected by concomitant treatment with atorvastatin in patients with acute coronary syndromes (5).

There is growing evidence that isoforms of cytochrome P450 besides CYP3A4 contribute to the conversion of clopidogrel into the active metabolite. Recently, experimental (25) and clinical studies (26, 27) added robust evidence for the involvement of CYP2C19 in the formation of the active metabolite of clopidogrel. The antiplatelet effect of clopidogrel is significantly attenuated in patients carrying at least one *CYP2C19*2* allele (26, 27) which encodes for a cryptic splice variant resulting in no enzyme activity *in vivo* (28, 29).

Consistent with the lack of effect of concurrent treatment with either atorvastatin or simvastatin on the antiplatelet effects of clopidogrel, we did not find an impact of statin co-medication on the 12-month incidence of death and MI in the subgroup of patients with PCI. A similar result was obtained after inclusion of target lesion interventions into the aforementioned composite of adverse events.

Limitations

We did not determine plasma concentrations of the active metabolite of clopidogrel (R130,964) in this study. Thus, we cannot provide direct evidence that CYP3A4-metabolized statins did not affect the systemic availability of the $P2Y_{12}$ receptor inhibiting metabolite. The chemical instability of the metabolite

requires extensive pre-analytical precautions and rapid chemical derivatization after blood withdrawal which cannot be warranted in a study on large patient cohorts under clinical conditions. Data from a recent study investigating the antiplatelet effects of loading doses of clopidogrel 300 mg and prasugrel 60 mg in healthy subjects indicate, however, that antiplatelet effects of both thienopyridines are closely related to the systemic availability of the active metabolite (30).

Patients were not randomized to the various statin groups. Although there were no significant differences in baseline characteristics between patients treated with different statins, patients without lipid-lowering therapy differed with respect to their baseline characteristics from those being treated with statins. We can not completely exclude possible bias by various risk factors and patient history, although the multivariable adjustments models confirmed the primary analysis.

We included only patients receiving daily doses up to 40 mg of the CYP3A4-metabolized statins atorvastatin and simvastatin because only a minority of patients in our hospital was treated with the maximum approved daily dose of 80 mg for atorvastatin or simvastatin. Thus, we have to restrict the results of the present study to the most frequently used dosing range from 10 to 40 mg of atorvastatin/simvastatin per day.

The study was not powered to exclude a minor effect of CYP3A4-statins on clinical endpoints in our patients undergoing PCI (n = 553) or to differentiate between individual statins in this respect.

Conclusions

This study demonstrates that statins metabolized by CYP3A4 (atorvastatin, simvastatin) administered in daily doses up to 40 mg have no impact on early antiplatelet effects of a 600-mg load-

ing dose regimen of clopidogrel. Consistently, our study does not suggest that statins metabolized by CYP3A4 (atorvastatin, simvastatin) curtail the beneficial effects of clopidogrel in PCI.

Acknowledgements

Abbreviations

ACE, angiotensin converting enzyme; ADP, adenosine diphosphate; ESC/ACC, European Society of Cardiology/American College of Cardiology; CABG, coronary artery bypass grafting: CYP, cytochrome; EXCELSIOR, Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate; GP, glycoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-Coenzyme A; LV, left ventricular; MACE, major adverse cardiac events; MI, myocardial infarction; PCI, percutaneous coronary intervention; TLR, target lesion revascularization.

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