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A screening study of drug-drug interactions in cerivastatin users: an adverse effect of clopidogrel

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Abstract

An analysis of a case-control study of rhabdomyolysis was conducted to screen for previously unrecognized CYP2C8 inhibitors that may cause other clinically important drug-drug interactions. Cases of rhabdomyolysis using cerivastatin (n=72) were compared with controls using atorvastatin (n=287) between 1998–2001. The use of clopidogrel (OR 29.6; 95% CI, 6.1–143) was strongly associated with rhabdomyolysis. In a replication effort that used the FDA Adverse Event Reporting System (AERS), clopidogrel was used more commonly by rhabdomyolysis cases using cerivastatin (17%) than by rhabdomyolysis cases using atorvastatin (0%, OR infinity; 95% CI = 5.2-infinity). Several medications were tested *in vitro* for their potential to cause drug-drug interactions. Clopidogrel, rosiglitazone and montelukast were the most potent inhibitors of cerivastatin metabolism. Clopidogrel and its metabolites also inhibited cerivastatin metabolism in human hepatocytes. These epidemiological and in-vitro findings suggest that clopidogrel may cause clinically important, dose dependent, drug-drug interactions with other medications metabolized by CYP2C8.

Keywords

rhabdomyolysis; statins; clopidogrel; adverse drug reaction; drug-drug interaction prediction; 2-oxo-clopidogrel; acyl glucuronide

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INTRODUCTION

The clinical trials required for drug approval are often small short-term studies, and these pre-approval trials usually exclude patients who have comorbid conditions or use multiple medications. For these reasons, uncommon adverse events and drug-drug interactions that increase the risk of adverse events may remain undetected prior to regulatory approval (1). Although *in vitro* studies of the drug metabolism that screen for drug-drug interactions are conducted in the early stages of drug development (2, 3), these approaches may lack the ability to predict drug-drug interactions in the setting of the complex physiology of patients. As a result, important drug-drug interactions are often discovered only after approval and sometimes late in the lifecycle of a drug.

The interaction between cerivastatin and gemfibrozil, a combination that markedly increases the risk of rhabdomyolysis, was not reported by the manufacturer until two years after the initial marketing of cerivastatin (4). In a population-based cohort study, the risk of rhabdomyolysis with cerivastatin monotherapy was 10-fold higher than with the use of other statins; with concurrent gemfibrozil use the risk was increased 50-fold (5). In a pharmacokinetic crossover trial of healthy volunteers, gemfibrozil increased the area under the plasma concentration-time curve (AUC) of cerivastatin by 559% (6). Gemfibrozil inhibits not only the oxidative metabolism of cerivastatin through cytochrome P450 enzyme (CYP) 2C8, but also hepatic transport through organic anion transporter protein (OATP) 1B1 (7–9). These pharmacokinetic effects of gemfibrozil are consistent with the compelling epidemiologic evidence of a drug-drug interaction.

In an effort to predict clinically important drug-drug interactions, *in vitro* assays that employ recombinant enzymes, human liver microsomes and human hepatocytes have been developed to screen for the inhibition of drug metabolism and drug transport (3, 10, 11). For instance, Walsky *et al.* used expressed enzymes and human liver microsomes to evaluate more than 200 commonly-used medications for their potential to inhibit CYP2C8-mediated metabolism (12). In an analogous fashion, it is possible to use epidemiological case-control studies of adverse drug reactions to screen directly for clinically important drug-drug interactions.

In this case-control study of cerivastatin use and rhabdomyolysis, we evaluated the use of various medications to identify potential inhibitors of CYP2C8. Medications that inhibit CYP2C8 metabolism may result in clinical drug-drug interactions with other medications that, like cerivastatin, rely on CYP2C8 metabolism for their clearance. As a replication effort, we evaluated medication use in cases of rhabdomyolysis using either cerivastatin or atorvastatin reported to the FDA Adverse Event Reporting System (AERS). Finally, to evaluate whether inhibition of CYP2C8 may account for some of the potential drug-drug interactions identified in the case-control study, we used CYP Supersomes® and human hepatocytes to perform a series of *in vitro* experiments. According to the FDA guidance on the conduct of drug interaction studies (2), we determined the $[I]/K_i$ ratio ($[I]$ = concentration of circulating inhibitor, K_i = *in vitro* inhibition constant). Changes in AUC were calculated from pharmacokinetic parameters to assess the likelihood that any observed inhibition *in vitro* may result in a clinical drug-drug interaction (13).

RESULTS

Case-control study

The available control group included 287 atorvastatin-using older adults from the Cardiovascular Health Study (CHS), so this drug interaction analysis included only the 72 age-matched cases who were 70 years or older, and excluded the other 143 cases who were

part of the original rhabdomyolysis study (14). Of the 72 cases, 92% were hospitalized, 39% percent developed renal failure, 14% required hemodialysis, and 3% died. The median peak creatine kinase (CK) level was 31,390 U/L (range 2,989–720,000 U/L) and the median dose of cerivastatin used at onset of rhabdomyolysis was 0.4mg/day (range 0.2–1.6mg/day). Cases were slightly younger than controls (mean age 76 vs. 80 years), and the prevalence of most comorbid conditions was slightly higher in cases than in controls (Table 1).

We evaluated 37 prescription medications that were used by at least 4% of cases (Table 2). As expected, the prevalence of gemfibrozil use in rhabdomyolysis cases was higher than in controls (32% vs. 0%; OR infinity; 95% CI, 25.0-infinity). Six other medications were also associated with rhabdomyolysis: fluoxymesterone (prevalence 8% in cases vs. 0% in controls; OR infinity; 95% CI, 4.4-infinity), clopidogrel (OR 29.6; 95% CI, 6.1–143), rosiglitazone (OR 19.8; 95% CI, 1.0–402), lansoprazole (OR 5.7; 95% CI, 1.3–24.0), rofecoxib (OR 4.9; 95% CI, 1.1–20.8), and propoxyphene (OR 4.8; 95% CI, 1.7–13.9). Restriction of the analysis to nonusers of gemfibrozil resulted in a smaller sample size, wider confidence intervals and, for some drugs, larger odds ratios. For clopidogrel, for instance, the OR increased from 29.6 (95% CI, 6.1–143) to 47.8 (95% CI, 12.5–182). Adjusting for use of the other medications identified as potential inhibitors, the OR for clopidogrel increased to 38.9 (95% CI 8.7–175.1, $P = 1.8 \times 10^{-6}$). Restriction to “severe rhabdomyolysis” cases with peak CK levels greater than 40 times the upper limit of normal (N=65) resulted in odds ratios similar to those from the primary analysis (Table S1).

FDA AERS

During identical periods of reporting, 594 rhabdomyolysis cases used cerivastatin and 75 cases used atorvastatin (Table 3). The odds ratio for concomitant gemfibrozil use in cerivastatin users vs. atorvastatin users was 24.6 (95% CI, 8.1–74.5). The reported prevalence of concomitant clopidogrel use was 17% in cerivastatin users and 0% in atorvastatin users (OR infinity; 95% CI, 2.6-infinity). The reported prevalence of clopidogrel use in cerivastatin cases was even higher when the analysis was restricted to aspirin users (29%; OR infinity; 95% CI, 1.2-infinity), or to nonusers of gemfibrozil (31%; OR infinity; 95% CI, 2.1- infinity). There was little evidence in AERS of an association for the other five medications evaluated (Table 3).

Inhibition of cerivastatin metabolism *in vitro*

Seven medications identified as potential inhibitors in the case-control study and 9 additional medications that did not appear to be inhibitors (controls) were selected for an *in-vitro* evaluation. The metabolites of clopidogrel, the strongest potential inhibitor identified in the case-control study, were also studied. The likelihood of a clinical drug-drug interaction was assessed using $[I]/K_i$ ratios. The maximal systemic total plasma concentration (C_{max}) from the literature served as the inhibitor concentration $[I]$, and K_i -values were determined in CYP2C8 Supersomes[®]. C_{max} was used as $[I]$ rather than $C_{max,u}$ because an *in vivo* interaction with gemfibrozil, a known potent inhibitor of CYP2C8 and the cause of a clinical drug-drug interaction with cerivastatin, could only be predicted *in vitro* with C_{max} (8).

Montelukast and rosiglitazone had $[I]/K_i$ ratios > 1 , and six additional medications other than clopidogrel had $[I]/K_i$ ratios between 0.1 and 1 (Table 4). For clopidogrel, at both the daily dose (75 mg) and loading dose (600mg), the $[I]/K_i$ ratio was < 0.1 . For clopidogrel carboxylic acid, a possible drug-drug interaction ($[I]/K_i = 0.14$) was predicted for the 75 mg dose, and a likely interaction was predicted for the 600mg dose ($[I]/K_i = 1.65$). Clopidogrel and 2-oxo-clopidogrel, though not predicted to cause drug-drug interactions on account of their low plasma concentrations, were both nonetheless potent *in vitro* inhibitors.

Because CYP3A4 accounts for roughly 40% of cerivastatin metabolism, mainly in the form of M-1 (8), the inhibition of cerivastatin metabolism by clopidogrel and metabolites in CYP3A4 Supersomes[®] was also assessed. In this experiment (Table 5), clopidogrel was a potent inhibitor of M-1 formation, and this inhibition was nearly as effective as clopidogrel's inhibition of M-23 formation in CYP2C8 Supersomes (Figure 1, Table 5).

The potential for drug-drug interactions with clopidogrel was also assessed in human cryopreserved hepatocytes, a complex system mimicking hepatic phase 1 and phase 2 drug metabolism and transport. Strong inhibitors of M-23 formation included carboxylic acid (76% $\pm$ 7%), *S*-(+)-clopidogrel (50% $\pm$ 11%), and 2-oxo-clopidogrel (43% $\pm$ 6%) (Table 5). Compared with inhibition of M-23 formation, inhibition of M-1 formation was less pronounced for clopidogrel and all metabolites evaluated (Table 5). Using M-23 as a marker for CYP2C8 activity and M-1 as marker for CYP3A4 activity, a 1.30-fold increase in AUC at a 75 mg dose and a 2.99-fold increase at a 600mg dose was predicted. The use of published values of maximal plasma concentrations for clopidogrel and the clopidogrel acid suggests a linear association between predicted AUC_i/AUC and dose (Figure 2).

DISCUSSION

In this case-control study of rhabdomyolysis designed to screen for novel drug-drug interactions, we confirmed that gemfibrozil is a potent inhibitor and identified another half-dozen drugs that may influence the clearance pathways of cerivastatin. Restricting the analysis to non-users of gemfibrozil affected most estimates only marginally. The most pronounced association was with clopidogrel (OR 29.6; 95% CI, 6.1–143). It remained after the exclusion of gemfibrozil users (OR 47.8; 95% CI, 12.5–182), and was replicated in the analysis of rhabdomyolysis cases from FDA AERS (OR infinity; 95% CI, 2.6-infinity). The *in vitro* evaluation confirmed that several medications identified in the case-control study are possible or likely inhibitors of CYP2C8. The use of CYP Supersomes and human hepatocytes demonstrated that clopidogrel and its metabolites are potentially potent inhibitors of CYP2C8 and CYP3A4. These in-vitro findings are consistent with the strong cerivastatin-clopidogrel interaction observed in the epidemiologic study.

In a 2002 report, the European Medicines Agency (EMA) Committee for Proprietary Medicine Products reviewed the evidence on the risk of rhabdomyolysis with cerivastatin and potentially interacting medications, which consisted of spontaneous adverse event reports (15). Twenty out of 546 (4%) rhabdomyolysis cases associated with cerivastatin use reported to the World Health Organization and 246 out of 1579 (16%) cases reported to Bayer were concomitant users of clopidogrel. Although these results are suggestive, the lack of controls, which precluded estimation of the magnitude of the increased risk, the uneven quality of the spontaneous adverse event reports, and the absence of findings from *in-vitro* studies left the status of early observations about this drug-drug interaction uncertain.

Structural differences in the probe substrates used in *in vitro* studies may influence the findings. In a screening study for CYP2C8 inhibitors by Walsky *et al.*, the predicted drug-drug interaction with clopidogrel was weak using amodiaquine as a probe (12). In previous reports that use paclitaxel or amodiaquine as probe substrates, neither clopidogrel (16) nor its metabolites (17) were strong inhibitors of CYP2C8. In contrast, the CYP2C8 metabolism of cerivastatin, an acidic drug, was influenced most by the acidic metabolites of clopidogrel. For CYP2C8-inhibition, for instance, the IC₅₀-values with cerivastatin as the substrate were 4-fold lower for the clopidogrel carboxylic acid and 85-fold lower for the glucuronide than with amodiaquine as the substrate. The findings of these *in vitro* experiments were consistent with those of the epidemiological studies.

The concomitant use of several other medications was associated with rhabdomyolysis in this case-control study. Rosiglitazone, a thiazolidinedione used to treat diabetes mellitus, has been shown *in vitro* to inhibit CYP2C8 with an associated $[I]/K_i$ ratio of 0.1–0.3 (18). Weak inhibition of OATP1B1 by rosiglitazone has been demonstrated *in vitro* as well (19). Using cerivastatin as a substrate, we identified rosiglitazone as a potent inhibitor of CYP2C8, and the $[I]/K_i$ ratio of 1.26 suggests a high likelihood of a clinical drug-drug interaction. Concomitant use of lansoprazole, rofecoxib, and propoxyphene also increased the risk of rhabdomyolysis in the case-control study. The screening study by Walsky et al. failed to identify any of these medications as potential CYP2C8 inhibitors, and neither did our *in vitro* evaluation. Fluoxymesterone, a drug used to treat male hypogonadism and female breast cancer (20, 21), was also associated with rhabdomyolysis. However, none of the 594 cerivastatin-associated rhabdomyolysis cases in AERS were users of fluoxymesterone, and our *in vitro* evaluation showed no potential for a drug-drug interaction.

Strengths of this case-control study include the successful recruitment of subjects with a rare adverse event, validation of the case diagnosis, and detailed ascertainment of medication use and indications for use in both groups. Cerivastatin and atorvastatin were first marketed at about the same time and had the same indications, and the AERS analysis provided further evidence of the interaction with clopidogrel use. The findings for clopidogrel were so strong that confounding and selection bias are not likely to be reasonable alternative explanations (22).

In this case control study, the control group, which consisted of atorvastatin users in a cohort study, was not optimal. Because rhabdomyolysis is a rare event and the prevalence of cerivastatin use was low during its market life, it was not possible to identify a single population from which cases and controls could be sampled with adequate power to screen for drug-drug interactions. For example, a cohort study of over 250,000 statin users from 11 geographically dispersed health plans identified only 24 cases of hospitalized rhabdomyolysis, which is one-third the size of the case group in our study (5). Also, the methods of data collection were not identical for cases and controls, and cerivastatin users and atorvastatin users may have differed in other unmeasured ways that affected the likelihood of their use of other medications. The recruitment of cases through attorneys may have resulted in a sample of more serious cases, and persons recruited from attorneys across the entire U.S. and Canada may differ from those sampled as controls from just four areas of the U.S. The sample size was sufficiently small that the study had low power to detect associations, with the result that some potential drug-drug interactions may have been missed. The design of this study, which includes only statin-users, does not distinguish whether concomitantly used medications increase the risk of rhabdomyolysis through an interaction or through a direct toxic effect on muscles, although neither clopidogrel nor the other medications identified as potentially interacting with cerivastatin are known to have a toxic effect on muscle, either *in vitro* or in a clinical setting. Finally, based on the *in vitro* I/K_i determinations, it would have been difficult to predict a significant interaction between clopidogrel and cerivastatin. The data provide supportive evidence of an interaction in the context of the strong epidemiological findings.

Several medications identified as potential inhibitors in the epidemiologic study were not confirmed in the *in vitro* evaluation. There are several potential explanations. First, some were not replicated in the AERS analysis. Second, as with clopidogrel, consideration of metabolites may be necessary to fully understand the inhibitory potential of the medication. Third, with the exception of clopidogrel, the *in vitro* model presented here did not consider all relevant clearance pathways of cerivastatin, such as CYP3A4 and OATP1B1 transport. Fourth, time-dependent inhibition of CYP2C8 and CYP3A4 was not considered in the prediction models, but could increase the estimated potential for a clinical drug-drug

interaction. Fifth, the role of CYP3A4 inhibition may have been underestimated as it is important in secondary metabolism, particularly the clearance of the cerivastatin lactone (23).

Published total plasma concentrations for specific doses of medications were used in our calculations, but the actual doses used clinically can be much higher and would increase the probability of a clinical drug-drug interaction. Although, for instance, the dose of 5mg was used to estimate the plasma concentration of glyburide, doses as high as 20mg/day are used. Also, a higher concentration in the portal vein can also be expected for some drugs, and the AUC_i/AUC -ratio can be further elevated in the presence of cirrhosis or renal impairment (24). Additionally, the weak evidence of CYP2C8 and CYP3A4 inhibition by clopidogrel carboxylic acid in Supersomes suggests that the marked inhibition of cerivastatin metabolism in hepatocytes may be in part the result of transporter inhibition.

Although cerivastatin was withdrawn from the market several years ago, these findings are relevant for other medications cleared through similar pathways. Amiodarone, an antiarrhythmic drug with thyroid, pulmonary, and hepatic toxicity, is metabolized by both CYP2C8 and CYP3A4 at therapeutic concentrations, and genetic variants of CYP2C8 decrease the clearance of this drug (25, 26). Other clinically important drugs metabolized primarily by CYP2C8 and CYP3A4 include imatinib (27), repaglinide (28), montelukast (29), pioglitazone (30) and carbamazepine (31). Using a case-control study of a rare adverse drug reaction to screen for novel drug-drug interactions, we have identified clopidogrel as a potentially strong inhibitor of the drug transport and metabolic pathways used by cerivastatin, and confirmed the role of CYP2C8 in this interaction. Further studies are needed to determine whether clopidogrel causes clinically important drug-drug interactions with other medications.

METHODS

Case control study

Cases were recruited through attorneys who had represented cerivastatin users with rhabdomyolysis. Because cerivastatin comprised a small fraction of statin use during its market life (March 1998-August 2001), it was not practicable to assemble a broad sample of users of cerivastatin who did not develop rhabdomyolysis as a basis for comparison (32). Instead older adults from CHS using atorvastatin, first marketed in February 1997, served as the control group, which was used to ascertain the expected frequencies of use of other medications (33). To match the age distribution of the available controls, we restricted the case group to persons aged 70 years and older. The recruitment of case subjects and use of CHS subjects as control subjects was approved by the University of Washington Institutional Review Board.

Case subjects who participated in the study were demographically similar to those who did not. For consenting cases, study staff conducted a telephone interview and obtained copies of medical records from attorneys, physicians, and hospitals. Trained abstracters used medical records to validate rhabdomyolysis events and collect information about participants' medical history. Rhabdomyolysis was defined as muscle pain or weakness, and a creatine kinase level > 10 times the upper limit of normal. The index date was the date of diagnosis.

The CHS is a prospective cohort study of risk factors for coronary heart disease and stroke in 5888 adults aged 65 or older. CHS design and recruitment have been described elsewhere (34, 35). Subjects were followed with annual visits or telephone calls that assessed medical conditions, medication use, hospitalizations, and cardiovascular events. CHS participants

were selected as controls if they used atorvastatin at an annual contact between 1998 and 2001 and if they were free of any inpatient diagnostic code for rhabdomyolysis in the year before their qualifying statin-use visit, called their index date. Controls were frequency matched to cases by sex at a ratio of approximately four per case.

Prescription medication use in cases was ascertained from medical and pharmacy records. In controls, prescription medication use was ascertained at annual study visits by interviewers who transcribed information from labels of prescription drug bottles (36). In both cases and controls, information on medical comorbidities including hypertension, diabetes, myocardial infarction, angina, previous angioplasty or coronary artery bypass surgery, congestive heart failure, stroke, transient ischemic attack, peripheral vascular disease, claudication, venous thromboembolism, and asthma was ascertained from medical records and from patient interviews. Treated diabetes mellitus was defined as the use of any antihyperglycemic medication, and treated hypertension was defined as the use of any drug used to treat hypertension in a patient who also had a diagnosis of hypertension. Cardiovascular disease was defined as a previous diagnosis of myocardial infarction, angina, stroke, transient ischemic attack, or peripheral vascular disease, or prior angioplasty or coronary artery bypass surgery.

Analysis of each medication was restricted to subjects with relevant medical conditions that define indications for the medication. For example, the analysis of clopidogrel was restricted to patients with evidence of prior coronary artery disease, stroke, or peripheral vascular disease, the approved indications for clopidogrel during the period studied. After restriction, medications used in at least 4% of cases were evaluated. In the primary analysis, odds ratios and 95% confidence intervals (CI) were estimated by logistic regression fit with robust standard errors and adjusted for age, gender, race, and index year. When the prevalence of medication use was zero in the control group, 95% CIs for odds ratios were estimated using exact methods (37, 38). Analyses were performed with STATA version 11.0 (StataCorp, College Station, TX). Because gemfibrozil use was uncommon among controls, it could not be included as a covariate in multivariable regression. In a secondary analysis, the analysis was repeated for each medication after the exclusion of gemfibrozil users. Additionally, the analysis was repeated restricting to “severe rhabdomyolysis” cases with peak creatine kinase levels > 40 times the upper limit of normal.

FDA AERS

The FDA AERS was used to further evaluate positive findings from the case-control study. For medications associated with rhabdomyolysis in the case-control study at a p value of < 0.05, the reported prevalence of use in cases of rhabdomyolysis using cerivastatin was compared with the reported prevalence of use in cases of rhabdomyolysis using atorvastatin. Cerivastatin is metabolized primarily by CYP2C8, while atorvastatin is metabolized primarily by CYP3A4 (11). This approach assumes that the drug of interest in the potential interaction does not inhibit both CYP2C8 and CYP3A4 to the same extent (39).

Reports of rhabdomyolysis in users of cerivastatin from date of FDA approval (June 26, 1997) to date of market removal (August 8, 2001) were included, as were reports of rhabdomyolysis in users of atorvastatin from date of FDA approval (December 17, 1996) through an identical period (January 9, 2001). Duplicates identified by age, event date, and concomitant medication use in the AERS database were excluded. Odds ratios and 95% CIs for each medication were estimated with logistic regression fit with robust standard errors and adjusted for age, gender, and time between FDA approval and the event date. Time from FDA approval to event date, rather than calendar year, was adjusted for because spontaneous adverse event reporting rates are typically highest during the earliest period of marketing, and cerivastatin and atorvastatin were approved in different years (40). When the prevalence

of medication use was zero in either group, 95% CIs for odds ratios were estimated using exact methods. Analyses were repeated after the exclusion of gemfibrozil users. Because information on medical comorbidity was unavailable for restriction, the analysis of clopidogrel use was repeated among users of aspirin, a surrogate for the presence of an indication for clopidogrel use.

***In vitro* evaluation of drug-drug interactions**

Chemicals—All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. Cerivastatin sodium salt, hydroxy (M-23) and desmethyl cerivastatin (M-1) were purchased from Toronto Research Chemicals (North York, ON, CA). Irbesartan, rofecoxib, pioglitazone, montelukast, 2-oxo-clopidogrel, *S*-(+)-clopidogrel and its *S*-(+)-carboxylic acid were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). CYP Supersomes[®] were purchased from BD Bioscience (San Jose, CA). Cryopreserved hepatocytes (H0737, 52 yrs, Hu1037, 57 yrs, Hu4152, 50 yrs, all female) were purchased from cellzdirect (Invitrogen inc., Carlsbad, CA).

Inhibition of cerivastatin metabolism in CYP2C8 and CYP3A4 Supersomes, and cryopreserved hepatocytes—For IC₅₀ measurements, CYP2C8 and CYP3A4 Supersomes were incubated for 5 min at 37° C and a cerivastatin concentration of 5 μM and 1 mM NADPH with the addition of inhibitors at 1, 3, 10, and 100 μM final concentrations (0.1% DMSO, 0.9% methanol). Reactions were quenched with acetonitrile containing 0.5 μM fluvastatin as internal standard, vortexed and centrifuged for 10 min at 4,000 rpm and 10 μL injected. Incubations with cryopreserved hepatocytes were started 24 hours post seeding in maintenance buffer including inhibitor. A K_m was measured for M-23 and M-1 formation (concentration range 0.2 to 50 μM, M-23: K_m 7.2 ± 1.2 and M-1: K_m 16.4 ± 1.7) at the shortest reasonable incubation time of 4 h. Inhibition experiments were performed at 5 μM cerivastatin concentration for 4 hours and the supernatant quenched and worked up as described under the IC₅₀ determination. For further details see Supplementary Information.

Detection of cerivastatin, clopidogrel and its metabolites—Metabolites and parent of clopidogrel and cerivastatin were separated on an Agilent Zorbax XDB C8-column (2.1 μm, 5 cm) and quantified on a Sciex API4000 LC/MS/MS (Applied Biosystems) in multireaction mode. For details see Supplementary Information.

Analysis—Apparent Michaelis-Menten constants K_m and k_{cat} were derived after nonlinear regression analysis (Sigma Plot 2004 Windows version 9.0, Systat Software, Chicago, IL). Percent inhibition was calculated for each reaction by correcting the potential solvent inhibition from the control assay. IC₅₀ values were derived by fitting the logarithm of the inhibitor concentration to the percent conversion values (one-site binding model for competitive inhibition). K_i values were derived by the equation $K_i = IC_{50}/2$ at the K_m (5 μM) or estimated using equation 1: ($K_i = ([I]/i - [I]) / (1 + [S]/K_m)$), (41), with fraction of inhibition i , inhibitor concentration $[I]$, cerivastatin concentration $[S]$, K_m Michaelis-Menten constant for cerivastatin metabolism) (42). IC₅₀ or K_i data are reported as the mean ± standard deviation. For *in vivo* values for $[I]$, the total systemic plasma concentration (C_{max} , bound and unbound) was used to estimate maximum possible drug-drug interaction and compared to the use of plasma concentration unbound ($C_{max\ u} = C_{max} \times f_u$, with f_u = fraction unbound in plasma). Administered drug doses were obtained from literature. The AUC_i/AUC was calculated using the fraction of metabolism by CYP2C8 and CYP3A4 and the inhibition of clopidogrel and its metabolites on the corresponding enzyme (13), assuming a $f_{m,CYP2C8} = 0.61$ and $f_{m,CYP3A4} = 0.37$, (8) with the K_i for M-23 as marker for CYP2C8 inhibition and for M-1 as marker for CYP3A4 inhibition as described in equation 2: $AUC_i/AUC = 1 / (f_{m,CYP} / ([I]_{in\ vivo} / K_i) + (1 - f_{m,CYP}))$, (43). Inhibition and control experiments were compared

using paired t-test (GraphPad Prism version 4.0, GraphPad Software Inc., San Diego, California, USA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Effect of clopidogrel and metabolites on percent formation of **A:** M-23 by CYP2C8 Supersomes, and **B:** M-1 by CYP3A4 Supersomes.

Figure 2.

Linear regression of predicted AUC_i/AUC and dose (C_{max} from references 6, 21)). $AUC_i/AUC > 1.25$ and > 2 suggest weak and moderate drug-drug interaction (51).

Table 1

Demographic and Clinical Characteristics of Cases and Controls

	Cases n=72		Controls n=287	
	Mean(SD)	Range	Mean(SD)	Range
Age, mean (SD)	76 (5)	70–90	80 (3)	74–90
	Count	Percent	Count	Percent
Male	23	32%	91	32%
White	67	93%	252	88%
Treated hypertension	50	69%	202	70%
Treated diabetes	14	19%	58	20%
Myocardial infarction	16	22%	62	22%
Angina	32	44%	111	39%
Angioplasty	25	35%	38	13%
CABG surgery	14	19%	41	14%
Stroke	12	17%	32	11%
Claudication	10	14%	20	7%
Cardiovascular disease *	48	67%	137	48%
Congestive heart failure	10	14%	45	16%
Atrial fibrillation	14	19%	33	11%
Asthma	14	19%	39	14%

CABG = coronary artery bypass grafting.

* Defined as the presence or history of myocardial infarction, angina, angioplasty, CABG surgery, stroke, or claudication.

Table 2

Medication Use and Association with Rhabdomyolysis.

	Restriction	Cases* n=72	Controls* n=287	Gemfibrozil Users Included			Gemfibrozil Users Excluded		
				OR	95% CI	P	OR	95% CI	P
Alendronate	-	8%	7%	1.3	0.5–3.4	0.653	1.0	0.3–3.1	0.986
Allopurinol	-	6%	2%	3.5	0.8–14.9	0.090	2.0	0.2–20.0	0.554
Amitriptyline	-	6%	2%	2.0	0.5–9.0	0.343	2.0	0.3–12.6	0.465
Amlodipine	HTN	28%	15%	2.3	0.9–5.5	0.074	1.9	0.7–5.0	0.199
Aspirin	CVD	60%	71%	0.6	0.3–1.3	0.179	0.8	0.3–1.9	0.579
Atenolol	HTN	24%	11%	1.9	0.7–4.8	0.208	2.2	0.8–5.9	0.125
Celecoxib	-	7%	7%	1.3	0.4–3.8	0.683	1.6	0.5–4.9	0.384
Clopidogrel	CVD	50%	8%	29.6	6.1–143	2.4×10 ⁻⁵	47.8	12.5–182	1.6×10 ⁻⁸
Digoxin	AF, CHF	35%	26%	1.3	0.4–4.9	0.688	0.3	0.1–1.7	0.169
Diltiazem	HTN	10%	6%	2.2	0.5–9.3	0.276	2.4	0.5–10.8	0.254
Enalapril	HTN	4%	5%	1.2	0.2–7.0	0.861	1.4	0.3–8.3	0.689
Furosemide	CHF	20%	42%	0.7	0.1–3.2	0.629	0.7	0.0–9.5	0.755
Gemfibrozil	-	32%	0%	inf	25.0–inf [†]	1.3×10 ⁻¹⁵	-	-	-
Glimepiride	DM	21%	12%	3.4	0.5–22.5	0.206	2.0	0.3–14.6	0.508
Glipizide	DM	29%	19%	3.7	0.7–19.7	0.120	2.4	0.3–22.9	0.440
Glyburide	DM	36%	41%	0.2	0.0–1.1	0.061	0.4	0.1–2.6	0.349
Fluoxymesterone	-	8%	0%	inf	4.4–inf [†]	1.2×10 ⁻⁴	inf	5.8–inf [†]	2.9×10 ⁻⁵
HCTZ	HTN	24%	21%	1.2	0.5–2.7	0.710	1.1	0.5–2.8	0.799
Irbesartan	HTN	6%	1%	4.8	0.8–29.8	0.094	4.4	0.6–34.6	0.156
Lansoprazole	-	11%	3%	5.7	1.3–24.0	0.018	4.7	1.3–17.5	0.020
Levothyroxine	-	26%	20%	1.7	0.9–3.2	0.100	1.5	0.7–3.2	0.251
Lisinopril	HTN	12%	19%	0.9	0.3–2.9	0.861	0.7	0.2–2.6	0.638
Losartan	HTN	6%	6%	0.9	0.2–4.2	0.905	0.9	0.2–5.2	0.925
Metformin	DM	29%	31%	0.6	0.1–3.3	0.553	0.2	0.0–3.3	0.242
Metoprolol	HTN	24%	18%	1.4	0.6–3.1	0.476	1.3	0.5–3.2	0.556
Montelukast	Asthma	9%	3%	2.8	0.3–25.0	0.360	inf	0–176 [†]	1.000
Oneprazole	-	4%	7%	0.5	0.2–1.6	0.263	0.6	0.1–2.2	0.404

	Restriction	Cases* n=72		Controls* n=287		Gemfibrozil Users Included			Gemfibrozil Users Excluded		
						OR	95% CI	P	OR	95% CI	P
Proglitazone	DM	7%		0%		inf	0.0-inf [†]	0.836	inf	0.1-inf [†]	0.543
Propoxyphene	-	8%		2%		4.8	1.7-13.9	0.003	2.7	0.6-11.2	0.174
Ramipril	HTN	4%		2%		0.9	0.2-3.8	0.848	1.1	0.2-4.5	0.938
Ranitidine	-	7%		3%		2.0	0.6-7.0	0.295	1.3	0.3-5.9	0.736
Rofecoxib	-	13%		4%		4.9	1.1-20.8	0.033	5.1	1.2-22.1	0.031
Rosiglitazone	DM	29%		5%		19.8	1.0-402	0.052	26.2	0.7-989	0.078
Triamterene	HTN	8%		5%		1.4	0.3-5.9	0.657	0.8	0.2-4.7	0.851
Valsartan	HTN	6%		5%		1.8	0.3-9.4	0.501	1.2	0.2-7.3	0.849
Verapamil	HTN	4%		4%		1.3	0.4-4.6	0.655	1.1	0.2-6.6	0.904
Warfarin	AF	29%		39%		0.5	0.1-2.4	0.372	0.2	0.0-1.2	0.080

AF = atrial fibrillation, CHF = congestive heart failure, CVD = cardiovascular disease, DM = diabetes mellitus, HTN = hypertension, inf = infinity.

* Prevalence of medication use in the restricted population.

[†] Exact methods used to obtain 95% confidence intervals and P values because there were no medication users in the control group.

Table 3

Medication Use in Rhabdomyolysis Cases from FDA Adverse Event Reporting System

	Cerivastatin n=594		Atorvastatin n=75		Gemfibrozil Included			Gemfibrozil Excluded		
	Count	Percent	Count	Percent	OR	95% CI	P	OR	95% CI	P
Clopidogrel	101	17%	0	0%	Inf	2.6-inf*	0.002	inf	5.2-inf*	1.1×10 ⁻⁵
Clopidogrel [†]	41	29%	0	0%	Inf	1.2-inf*	0.037	Inf	2.1-inf*	0.003
Gemfibrozil	284	48%	5	7%	24.6	8.1–74.5	1.1×10 ⁻⁸	-	-	-
Fluoxymesterone	0	0%	0	0%	-	-	-	-	-	-
Lansoprazole	21	4%	1	1%	3.3	0.3–37.0	0.336	inf	0.4-inf*	0.334
Propoxyphene	9	2%	1	1%	1.0	0.2–4.9	1.000	2.3	0.4–12.0	0.316
Rofecoxib	5	1%	1	1%	0.2	0.0–1.6	0.147	0.5	0.1–2.9	0.412
Rosiglitazone	14	2%	1	1%	0.7	0.1–6.1	0.772	0.6	0.1–7.3	0.715

* Exact methods used to generate confidence intervals because prevalence of clopidogrel use was 0% among atorvastatin cases.

[†] Restricted to aspirin users only.

Pharmacokinetic parameters, inhibitory effect (K_i) for M-23-formation using CYP2C8 Supersomes, and prediction of clinical drug-drug interactions (II/ K_i).

Table 4

Drug	Oral Dose (mg)	Ref.	C_{max} (ng/mL)	f_u	IC_{50} (μ M)	K_i (μ M)	II/ K_i ⁶ (II = $C_{max,u}$)	II/ K_i ⁶ (II = C_{max})
Amlodipine	10	44	18	0.07	4.0 $\pm$ 0.7	2.0	<0.01	0.02
Celecoxib	200	44	705	0.03	5.4 $\pm$ 0.5	2.7	0.02	0.68
Diltiazem ¹	120	44	151	0.22	124 $\pm$ n.d.	62	<0.01	0.01
Fluoxymestrone	100	45	19	0.04	16 $\pm$ 6	8.0	<0.01	0.01
Glyburide	5	44	105	0.002	6.7 $\pm$ 2.2	3.4	<0.01	0.06
Irbesartan	50	44	1300	0.1	16 $\pm$ 3	8.1	0.04	0.37
Lansoprazole ²	15	44	248	0.03	19 $\pm$ 5	9.6	<0.0	0.07
Levothyroxine	0.6	12	591	0.01	4.6 $\pm$ 0.2	2.3	<0.01	0.33
Montelukast	10	44	542	0.01	0.02	0.05	0.18	18.5
Pliglitazone	30	44	1500	0.01	16 $\pm$ 10	7.8	<0.01	0.54
Propoxyphene	65	46	100	0.2	18 $\pm$ 3	8.9	<0.01	0.03
Rofecoxib	25	44	320	0.13	14 $\pm$ 5	6.9	0.02	0.15
Rosiglitazone	8	44	598	0.002	2.7 $\pm$ 0.1	1.3	<0.01	1.26
Verapamil ^{1,3}	120	44	272	0.1	6.8 $\pm$ 2.9	3.4	0.02	0.18
Clotidogrel	75/600 ⁴	44, 47, 48	2/31.4	0.02	3.4 $\pm$ 0.6	1.7	<0.01/<0.01	<0.01/0.06
Clotidogrel acid	75/600 ⁴	47, 49	3000/34,394	0.06	136 $\pm$ 31	68	<0.01/ 0.1	0.14/1.65
2-oxo clotidogrel	600 ⁴	49	0.0488	0.02	4.2 $\pm$ 0.6	2.1	<0.01	<0.01
Clotidogrel acyl glucuronide ⁵					4.6 $\pm$ 0.6	2.3	n.d.	n.d.
Gemfibrozil	600	42	25,000	0.0064		14	0.07	7.1

For inhibition of M-1-formation, see supplementary information.

¹ mechanism-based inhibitor for CYP3A4;

² lansoprazole IC_{50} CYP3A4 6.5 μ M (50);

³ mechanism-based inhibitor for CYP2C8;

⁴ single loading clotidogrel dose in a population of individuals with coronary artery disease who underwent stent implantation (49);

⁵ the concentration of circulating clopidogrel carboxylic acid glucuronide is unknown.

⁶ An $[I]/K_i$ ratio > 1 suggests a highly likely, an $[I]/K_i$ ratio $0.1-1$ a possible, and an $[I]/K_i$ ratio < 0.1 remote possibility of an interaction.

Table 5

Inhibition of cerivastatin metabolite formation (M-23 and M-1) by clopidogrel and clopidogrel metabolites using CYP3A4 Supersomes and human cryopreserved hepatocytes.

Inhibitor	Cerivastatin metabolite	Supersomes			Hepatocytes ³		
		CYP2C8		CYP3A4	(n=3)		
		IC ₅₀ (μM)	K _i (μM)		% inhibition	K _i (μM)	
S-(+)-clopidogrel	M-23	3.4 ± 0.6	1.7	n.d.	50 ± 11 *	n.d.	2.3
	M-1	2.8 ± 0.8	1.4	4.7 ± 1.0	30 ± 6 *	3.7	18.9
S-(+)-clopidogrel carboxylic acid	M-23	136 ± 31	68	n.d.	76 ± 7 ***	n.d.	18.4
	M-1	107 ± 26	54	356 ± 38	35 ± 3 **	280	188
2-oxo-clopidogrel	M-23	4.2 ± 0.6	2.1	n.d.	43 ± 6 *	n.d.	3.9
	M-1	4.1 ± 0.2	2.1	3.1 ± 1.0	31 ± 5 *	2.4	50.8
clopidogrel acyl glucuronide	M-23	4.6 ± 0.6	2.3	n.d.	13 ± 2	n.d.	19.1
	M-1	3.7 ± 0.8	1.9	27 ± 5	5 ± 1	21	845

n.d. = not determined.

¹ reproduced from Tables 1 and S2;

² calculated from %-inhibition using equation 1;

³ inhibitor concentrations for hepatocyte experiments: S-(+)-clopidogrel 5 μM, S-(+)-clopidogrel carboxylic acid 100 μM, 2-oxo-clopidogrel 5 μM, clopidogrel acyl glucuronide 5 μM;

* p<0.05,

** p<0.01,

*** p<0.001.