Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

# Sotrastaurin single-dose pharmacokinetics in *de novo* liver transplant recipients

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**Keywords**
immunosuppression, liver transplantation, pharmacokinetics, protein kinase C.

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**Conflicts of Interest**
The authors have declared 'no conflicts of interest'.

Received: 3 September 2010
Revision requested: 7 October 2010
Accepted: 9 November 2010
Published online: 7 December 2010

doi:10.1111/j.1432-2277.2010.01196.x

## Summary

Sotrastaurin is a protein kinase C inhibitor in development for prevention of rejection after liver transplantation. In a pharmacokinetic study, 13 *de novo* liver transplant recipients received 100 mg sotrastaurin once between days 1–3 and once between days 5–8 post-transplant. Sotrastaurin absorption based on the area under the concentration-time curve (AUC) of total drug in blood (3544 ± 1434 ng·h/ml) was similar to that of healthy subjects in a previous study (4531 ± 1650 ng·h/ml). However, the sotrastaurin binding protein, α1-acid glycoprotein, was nominally higher in patients (1.07 ± 0.28 vs. 0.87 ± 0.16 g/l, $P = 0.13$) yielding a 60% lower AUC based on free drug versus that in healthy subjects (27 ± 13 vs. 62 ± 15 ng·h/ml, $P < 0.0001$). There was minor excretion of sotrastaurin in drained bile (1% of dose) consistent with the fact that sotrastaurin is extensively metabolized leaving little unchanged drug to excrete. In the first week after liver transplantation, sotrastaurin is bioavailable after oral administration. However, patients with elevated α1-acid glycoprotein levels may have lower free drug concentrations. Whether a higher dose of sotrastaurin is needed to compensate for this in the short-term after surgery will be addressed in future clinical trials.

## Introduction

Sotrastaurin (AEB071) is a novel immunosuppressant that blocks T-cell activation via inhibition of protein kinase C, integrating signaling pathways downstream of the T-cell receptor and the CD28 coreceptor. Sotrastaurin inhibits potently and specifically early T-cell activation (signal 1 and 2) but not T-cell proliferation (signal 3). Sotrastaurin is being developed for the prevention of acute rejection in solid organ allotransplantation in regimens with or without a calcineurin inhibitor. In contrast to calcineurin inhibitors, sotrastaurin selectively blocks a calcineurin-independent pathway pointing toward a differentiation in mode of action, and possibly side-effect profile. After oral administration, sotrastaurin reaches peak blood concentrations around 2–3 h postdose that subsequently decline with a half-life of about 6 h. It is highly protein bound (>98%) primarily to α1-acid-glycoprotein. Renal excretion of sotrastaurin in minimal (<2% of the dose) but metabolism is extensive and occurs primarily via CYP3A4. Only the minor metabolite *N*-desmethyl-sotrastaurin is pharmacologically active but exposure is low at blood concentrations <5% those of the parent compound.

Sotrastaurin has been investigated in preclinical animal transplant models [1], animal toxicology studies and single- and multiple-dose studies in healthy subjects. Initial clinical trials have been conducted in kidney transplantation [2] and psoriasis [3]. We describe herein the first assessment of sotrastaurin in liver transplantation. The first weeks after liver transplantation is a time of notable intrapatient pharmacokinetic variability for some orally administered drugs. This is especially true for several

commonly prescribed immunosuppressants such as ciclosporin, tacrolimus, and mycophenolic acid. Patients undergo a time-dependent change in organ function from that of pretransplant liver failure to normalization. This normalization includes an increase in synthetic function (production of plasma proteins) as well as clearance of bilirubin and xenobiotics. During this same period, organ preservation, cold ischemia time between organ procurement and engraftment, and the trauma of surgery may lead to significant alteration in oral drug absorption and/or metabolism. In addition, external bile drainage via a T-tube may compromise drug bioavailability if bile is important in solubilizing the drug before absorption from the gastrointestinal tract.

Before embarking on clinical trials in liver transplantation with sotrastaurin, we conducted this phase 1 study to evaluate the single-dose pharmacokinetics and acute tolerability of sotrastaurin in the first week post-transplant. Our intention is to combine the pharmacokinetic data from this single-dose study with existing information from phase 2 trials in renal transplantation to design a subsequent phase 2 dose-finding study for sotrastaurin in liver transplantation. The primary objective of the current study was to characterize the pharmacokinetics of sotrastaurin in liver transplant patients at the beginning and end of the first week post-transplant to determine if sotrastaurin is bioavailable after oral administration in these patients and whether exposure changed over this time period. The secondary objectives were to evaluate the tolerability of single-dose sotrastaurin; the biliary excretion of sotrastaurin and its active metabolite *N*-desmethyl-sotrastaurin; the relationship of sotrastaurin concentrations and α1-acid-glycoprotein concentrations (the protein to which sotrastaurin predominantly binds in plasma); and the pharmacokinetics of tacrolimus in the presence of sotrastaurin.

## Patients and methods

### Study design

This was a multicenter, open-label pharmacokinetic study planned for 12 *de novo* liver transplant patients receiving a tacrolimus-based immunosuppressive regimen. Patients received two single-doses of 100 mg sotrastaurin orally. The first dose was given once between days 1–3 post-transplant (period 1) and the second dose was given once between days 5–8 post-transplant (period 2). A patient's participation was complete with the last pharmacokinetic blood and bile collection 72 h after the sotrastaurin dose in period 2. The protocol was reviewed and approved by investigational review boards at each study center and the study was performed in accordance with the Declaration of Helsinki. Patients gave written informed consent to participate. This study was registered with ClinicalTrials.gov number: NCT00545259. We began enrollment on 3 October 2007 and the study was completed on 17 February 2008.

### Study population

We enrolled men and women, 18 years of age or older, who were recipients of a primary orthotopic liver transplant. The allograft was to be functioning at an acceptable level by 24 h post-transplant as determined by the investigator. The immunosuppressive regimen had to contain tacrolimus; whereas the use of mycophenolic acid or corticosteroids was optional. Women capable of becoming pregnant had to have a negative pregnancy test within 7 days before enrollment. We excluded patients meeting any of the following criteria: recipients of an ABO-incompatible transplant; recipients of a living donor or split-liver transplant; recipients of a liver with a cold ischemia time of >12 h; recipients of a liver from a donor after cardiac death; patients with a model for endstage liver disease (MELD)-score >35 in the month before transplantation; transplant of a marginal graft into a patient with a MELD-score >28 (defined as donor age >60 years, cold ischemia time >10 h, hypotension periods or inotropic support of the donor, intensive care unit stay >3 days, graft steatosis >30%); patients with acute fulminant hepatic failure (UNOS I, T1); patients with a positive HIV serology; patients with any past or present malignancy other than excised basal cell carcinoma or hepatocellular carcinoma; patients with a serum creatinine >4 mg/dl or on dialysis; patients who required the coadministration of drugs that are strong inhibitors or inducers of the CYP3A4; patients with a history of or a current clinically significant cardiac abnormality; patients with an absolute neutrophil count $<1.5 \times 10^9$/l, or absolute leukocyte count $<2.5 \times 10^9$/l, or platelets $<40 \times 10^9$/l; patients with severe active infection.

### Drug administration

Sotrastaurin was administered at a dose of 100 mg either orally or via a nasogastric tube. For patients who could take medications by mouth, sotrastaurin was given as a capsule with adequate water. For patients who could not take oral medications, the contents of a sotrastaurin capsule were suspended in 10–15 ml of tap water in a 30 ml dosing cup. The suspension was then drawn up into a 50 ml oral syringe. The dosing cup was rinsed with about 10 ml tap water and this was drawn up into the syringe as well. The total suspension was administered via the nasogastric tube. Thereafter, the nasogastric tube was flushed with 5–10 ml of water. In a given patient, whichever route of administration was used for the first dose was to be used for the second dose, if possible.

All patients were required to have tacrolimus in their immunosuppressive regimen dosed according to the center's standard practice and initiated within 12 h of transplantation. Corticosteroids and mycophenolic acid were optional. All other co-medications were allowed with the exception of strong CYP3A4 inhibitors or inducers.

### Clinical assessments

Pulse and blood pressure were recorded predose and then 2, 4, 6, and 24 h after each dose. Standard clinical laboratory parameters were assessed predose and then 24, 48, and 72 h after each dose. Electrocardiograms were recorded before and 4 h after each dose.

### Pharmacokinetics and bioanalytics

Pharmacokinetic venous blood samples were collected predose and then 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 16, 24, 36, 48, and 72 h postdose for measurement of sotrastaurin, *N*-desmethyl-sotrastaurin, and tacrolimus in whole blood. An additional blood sample was collected 3 h postdose for the determination of free (unbound) sotrastaurin and *N*-desmethyl-sotrastaurin in plasma. Externally drained bile was collected quantitatively over the time intervals, −1 to 0 h predose and then 0–2, 2–6, 6–24, 24–48, and 48–72 h postdose. The total bile volume was recorded in each time interval and a portion kept for analysis. Blood, plasma, and bile samples were frozen at −70 °C.

Sotrastaurin, *N*-desmethyl-sotrastaurin, and tacrolimus were determined in blood, plasma, and bile by a validated liquid chromatography method with tandem mass spectrometry (LC-MS/MS). The assay limit of quantification was 3 ng/ml for sotrastaurin and *N*-desmethyl-sotrastaurin and 1 ng/ml for tacrolimus.

### Analysis of plasma protein binding

The unbound fraction of sotrastaurin in plasma was estimated by equilibrium gel filtration as described by Soltes [4]. For this purpose a chromatography system with two 5 ml HiTrap desalting columns in series (GE Healthcare BioSciences, Uppsala, Sweden) including ultraviolet/radiometry detection systems was applied. A high-performance liquid chromatography column was preloaded with [$^{14}$C] sotrastaurin diluted in phosphate buffered saline at a nominal concentration of 5 ng/ml and then loaded with plasma samples diluted (1:2) with phosphate buffer saline. The unbound fraction of *N*-desmethyl-sotrastaurin was determined by means of equilibrium dialysis employing a Teflon 96-well block (HTDialysis, Gales Ferry, CT, USA) as described by Banker *et al.* [5] followed by the quantification of drug by LC-MS/MS.

### Pharmacokinetic and statistical evaluation

Standard noncompartmental pharmacokinetic parameters were calculated. For sotrastaurin these included the maximum drug concentration ($C_{max}$), the time of its occurrence ($t_{max}$), the area under the concentration-time curve extrapolated to infinity (AUC), and the elimination half-life ($t_{1/2}$). Given the lower *N*-desmethyl-sotrastaurin blood concentrations, only $t_{max}$, $C_{max}$, and AUC to the last blood sampling time [AUC(0−*t*)] could be calculated. For both analytes, $C_{max}$ and AUC based on free, nonprotein-bound drug were derived by multiplying the parameter by the fraction of analyte unbound in blood (fu,b). The latter was calculated as: fu,b = Cp/Cb × fu,p, where *C*p is the analyte concentration in plasma, *C*b is the analyte concentration in blood, and fu,p is the fraction of analyte unbound to proteins in plasma.

Pharmacokinetic parameters were summarized as mean ± standard deviation. Logarithm-transformed $C_{max}$ and AUC were evaluated in a linear mixed-effects model with *period* as a fixed effect and *patient* as a random effect. Relationships between pharmacokinetic parameters and α1-acid-glycoprotein levels were evaluated by graphical inspection and linear regression. To compare pharmacokinetic data from this study in patients to data in healthy subjects we chose a previous study that most closely resembled the current study. This involved six healthy subjects who received a single 100 mg dose of sotrastaurin and subsequently had whole blood concentrations of sotrastaurin and its metabolite determined along with protein binding measurements and α1-acid-glycoprotein levels (data on file). Pharmacokinetic parameters were logarithm-transformed and compared between the groups with unpaired *t*-tests.

## Results

### Patients and drug administration

Thirteen patients participated in the study. Characteristics of the patient population are summarized in Table 1. The transplanted organ in all cases was from a living donor. Twelve patients received both sotrastaurin doses and one patient received the first dose but was withdrawn from the study before period 2 because of a serious adverse event (described below).

Of the 13 patients who participated in the study, 11 patients provided evaluable pharmacokinetic data from both dosing days and two patients provided data from a single dosing day (one patient's samples were lost in transit, one patient was withdrawn from the study). Hence, there were 12 datasets for day 1–3 and 12 datasets for day 5–8. All administrations were by mouth (capsule) with the exception of two patients. One received the first dose

**Table 1.** Demographic and clinical characteristics of the study population.

| Characteristic | Day 1–3 | Day 5–8 |
|---|---|---|
| Gender (male/female) | 8/5 | – |
| Age (years) | 57.2 ± 7.0 | – |
| Weight (kg) | 84.8 ± 16.2 | –* |
| Body mass index (kg/m$^2$) | 28.0 ± 4.4 | –* |
| Total bilirubin (μmol/l) | 49 ± 57 | 87 ± 119 |
| AST (U/l) | 378 ± 399 | 55 ± 27 |
| ALT (U/l) | 428 ± 265 | 136 ± 61 |
| GGT (U/l) | 124 ± 79 | 260 ± 208 |
| Albumin (g/l) | 31 ± 4 | 31 ± 4 |
| Creatinine clearance (ml/min) | 84 ± 35 | 92 ± 44 |
| Tacrolimus dose (mg bid) | 4.4 ± 1.4 | 5.5 ± 2.3 |
| Prednisolone dose (mg/day) | 82 ± 22 | 39 ± 22 |

Values are mean ± SD.
*Only recorded at study entry.

via nasogastric tube and the second dose by mouth and the other received both doses via nasogastric tube. Pharmacokinetic parameters did not appear to differ by route of administration; hence, they were combined in the description below. All patients received tacrolimus; two patients received mycophenolic acid (for which no pharmacokinetic drug interaction was found in a dedicated study addressing this in healthy subjects [6]); and 11 patients received prednisolone. Average doses of tacrolimus and prednisolone are given in Table 1.

### Sotrastaurin pharmacokinetics based on total drug in blood

Figure 1 shows the mean concentration-time profiles of sotrastaurin and metabolite based on total concentrations in blood and Table 2 summarizes the corresponding pharmacokinetic parameters. At the first dosing occasion on days 1–3, $t_{max}$ generally clustered between 1 and 2 h postdose with three later values at 3, 4, and 9 h indicative of some delays in drug absorption. Later $t_{max}$ values were not necessarily associated with lower peak concentrations ($C_{max}$). $C_{max}$ clustered in the range 214–603 ng/ml with one outlier of 2010 ng/ml. Because of this outlier, the coefficient of variation for $C_{max}$ was 91% (without the outlier, 30%). AUC ranged 3.6-fold from 1697 to 6160 ng·h/ml with a coefficient of variation of 40%. Half-lives ranged from 4.1 to 15.7 h. After the second dose on days 5–8, the pharmacokinetics of sotrastaurin was similar to those after the first dose inasmuch as all statistical comparisons were nonsignificant.

The time to reach *N*-desmethyl-sotrastaurin peak concentrations ($t_{max}$) occurred shortly after the $t_{max}$ of sotrastaurin. Thereafter, metabolite blood concentrations were quantifiable generally to 9 or 12 h postdose which precluded the estimation of the terminal half-life. Metabolite total exposure was highly variable between patients with a coefficient of variation for AUC(0–t) of 98%. Exposure to the metabolite relative to that of the parent compound (metabolic ratio) averaged 4%. Metabolite $C_{max}$ and AUC were not significantly different at the second dose on days 5–8 compared with the first dose.

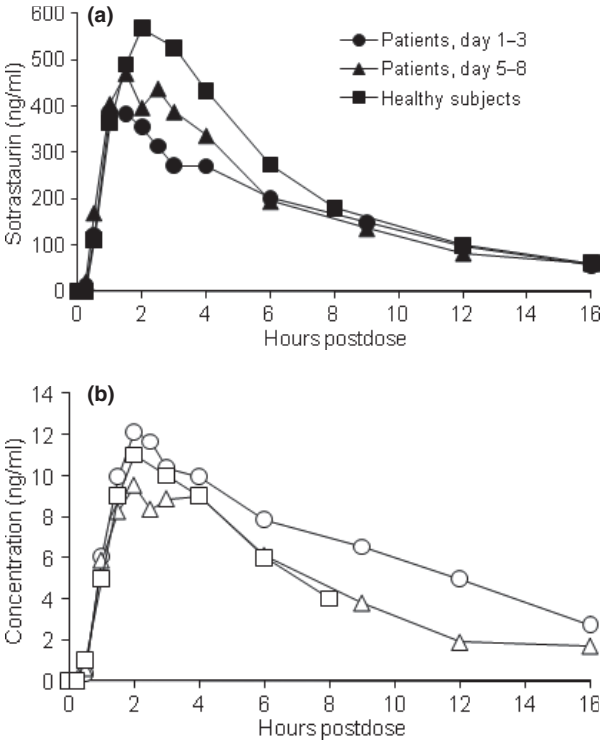


**Figure 1** Mean sotrastaurin (panel a) and *N*-desmethyl-sotrastaurin (panel b) blood concentration profiles after a single 100 mg dose in liver transplant patients assessed on day 1–3 (circles) and day 5–8 (triangles) post-transplant and compared with healthy subjects (squares) from a previous study. Bars are one standard deviation of the mean. The plots focus on the first 16 h postdose to facilitate visual comparison of the initial absorption patterns between groups although all concentration data to 72 h were used in the pharmacokinetic evaluation.

Sotrastaurin mean exposure in patients based on total concentrations in blood on days 1–3 as described above were similar to those of six healthy subjects in a previous study who received 100 mg and achieved a $C_{max}$ of 612 ± 117 ng/ml ($P = 0.09$), AUC of 4531 ± 1650 ng·h/ml ($P = 0.15$), and half-life of 9.2 ± 2.9 h ($P = 0.62$). With respect to *N*-desmethyl-sotrastaurin, the mean $C_{max}$ was similar in patients and healthy subjects (12 ± 4 ng/ml, $P = 0.77$) but mean AUC(0–t) was about twofold higher in patients relative to healthy subjects (58 ± 19 ng·h/ml, $P = 0.25$) against a background of high inter-subject variability in patients.

**Table 2.** Sotrastaurin pharmacokinetic parameters.

| | Sotrastaurin | | *N*-desmethyl-sotrastaurin | |
|---|---|---|---|---|
| Parameter | Day 1–3 | Day 5–8 | Day 1–3 | Day 5–8 |
| Total analyte | | | | |
| $t_{max}$ (h) | 1.8 (1–9) | 1.8 (1–6) | 2.5 (1.5–9) | 2.4 (1.4–6) |
| $C_{max}$ (ng/ml) | 530 ± 480 | 631 ± 471 | 14 ± 8 | 13 ± 7 |
| AUC (ng·h/ml) | 3544 ± 1434 | 3954 ± 3304 | 137 ± 134 | 102 ± 197 |
| $t_{1/2}$ (h) | 8.7 ± 3.5 | 9.5 ± 4.5 | – | – |
| Amount excreted in bile (mg) | 1.1 ± 0.6 | 0.7 ± 0.7 | 3.3 ± 2.2 | 1.9 ± 2.1 |
| Free analyte | | | | |
| Fraction free in blood (%) | 0.7 ± 0.2 | 0.7 ± 0.2 | 7.0 ± 1.4 | 5.7 ± 1.1 |
| $C_{max}$ (ng/ml) | 3.6 ± 2.6 | 3.8 ± 2.0 | 1.1 ± 0.5 | 0.7 ± 0.3 |
| AUC (ng·h/ml) | 27 ± 13 | 24 ± 19 | 11 ± 8 | 6 ± 10 |

Values are mean ± standard deviation except for $t_{max}$ which is median (range).
Free analyte refers to sotrastaurin or metabolite not bound to plasma proteins.
$T_{max}$ is time of peak concentration, $C_{max}$ is peak concentration, AUC is area under the concentration-time curve, $t_{1/2}$ is half-life, amount excreted in bile is over 72 h postdose.

### Sotrastaurin pharmacokinetics based on free drug in blood

Alpha1-acid-glycoprotein is the main plasma protein to which sotrastaurin binds. As concentrations of this acute phase protein can be altered in organ transplant patients, we included an assessment of free drug levels in our study. Patient's plasma levels of α1-acid-glycoprotein were generally high on both day 1–3 (1.07 ± 0.28 g/l) and day 5–8 (1.25 ± 0.37 g/l) relative to the laboratory normal range of 0.5–1.0 g/l and relative to the healthy subjects (0.87 ± 0.16). When the data from patients and healthy subjects were combined, there was a significant negative correlation between the α1-acid-glycoprotein level and the fraction of sotrastaurin free in whole blood (the matrix in which sotrastaurin is measured clinically) as illustrated in Fig. 2. The free fraction of sotrastaurin in blood in patients (0.7 ± 0.2%) was half that in healthy subjects (1.4 ± 0.3%). For *N*-desmethyl-sotrastaurin, which is weakly bound to α1-acid-glycoprotein, the mean free fraction in blood was higher in patients (7.0 ± 1.4%) than in healthy subjects (4.7 ± 1.0%).

As seen in Fig. 3, sotrastaurin AUC based on free drug was generally lower in patients on both assessment days compared with healthy subjects. The free AUC for sotrastaurin in healthy subjects was 62 ± 15 ng·h/ml compared with 27 ± 13 ng·h/ml in patients on day 1–3 ($P < 0.0001$). Hence, the free exposure for patients was about 60% lower, on average, and this difference was maintained over the course of the week. The free AUC(0–t) for *N*-desmethyl-sotrastaurin was 2.6 ± 0.6 ng·h/ml for healthy subjects. As summarized in Table 2, it was 4.2-fold higher in patients on day 1–3 ($P = 0.002$) and then normalized to healthy subject values by day 5–8 ($P = 0.47$).

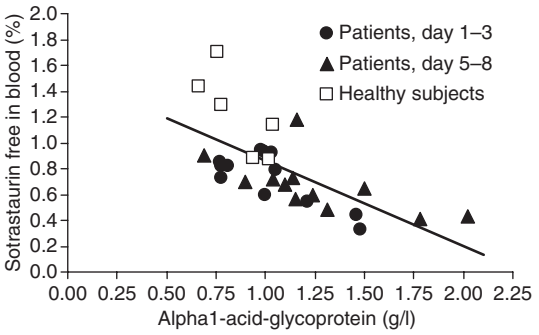


**Figure 2** Scatterplot of α1-acid-glycoprotein levels versus percent of sotrastaurin free (not protein bound) in blood obtained 3 h postdose. Designated are values from liver transplant patients assessed on day 1–3 (filled circles) and day 5–8 (filled triangles). Healthy subject data are from a previous study (open squares). Shown is the regression line with a slope –0.66 g/l/% and intercept 1.52% ($r^2 = 0.45$, $P < 0.001$).

### Sotrastaurin biliary clearance

While all 13 patients had external biliary drainage at both dosing occasions, missing or inaccurate records of drained bile volumes in some patients yielded evaluable data in 10 patients for each dosing occasion. The volume of bile drained externally per day in the 3-day period of collection after each dose was relatively stable on average. After dose 1 the consecutive volumes were 254 ± 150, 269 ± 118, and 318 ± 262 ml/day. After dose 2, the volumes were 352 ± 264, 338 ± 223, and 319 ± 225 ml/day. As the standard deviations relative to the means indicate, there was a wide range of volumes between patients from 30 to 940 ml/day. Biliary drug concentrations were highest in the 6–24 h fractional collection for both sotrastaurin and *N*-desmethyl-sotrastaurin. The metabolite

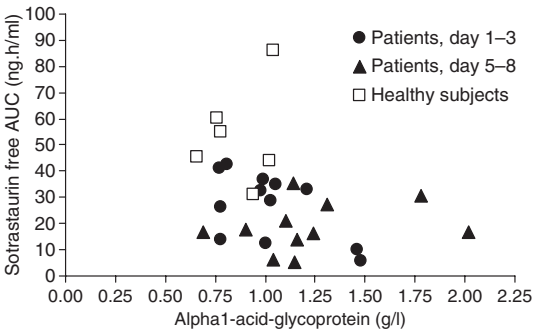


**Figure 3** Scatterplot of α1-acid-glycoprotein levels versus sotrastaurin area under the concentration-time curve based on free (not protein bound) blood concentrations. Designated are values from liver transplant patients assessed on day 1–3 (filled circles) and day 5–8 (filled triangles). Healthy subject data are from a previous study (open squares).

concentrations were generally higher than those of the parent compound. The corresponding pharmacokinetic parameters are summarized in Table 2. Around 1.1 mg of intact sotrastaurin and 3.3 mg *N*-desmethyl-sotrastaurin were excreted after the first dose. Both amounts were numerically lower after the second dose at 0.7 mg and 1.9 mg. All values exhibited high intersubject variability of >50%. Biliary clearance of intact sotrastaurin was negligible (around 0.3 l/h) which is consistent with the fact that sotrastaurin is extensively metabolized leaving little unchanged parent drug to be cleared in the bile. Biliary excretion of *N*-desmethyl-sotrastaurin was minor.

### Tacrolimus pharmacokinetics and biliary clearance

The tacrolimus dose given on the day of the first sotrastaurin assessment averaged 4.4 ± 1.4 mg twice-daily (range: 2–7) and the tacrolimus dose on the day of the second sotrastaurin assessment averaged 5.5 ± 2.3 mg twice-daily (range: 3–9.5). These doses are in the range usually used early after liver transplantation. On days 1–3 and days 5–8, trough levels were 7.9 ± 4.7 and 7.5 ± 3.7 ng/ml, $C_{max}$ was 14.4 ± 7.8 and 15.4 ± 3.4 ng/ml, and AUC(0−12) was 120 ± 68 and 118 ± 32 ng·h/ml. When all trough-AUC(0−12) data pairs were pooled over the study course, there was a significant positive correlation ($r^2 = 0.713$, $P < 0.001$).

The amount of tacrolimus excreted in bile was derived per day in the 3-day period of bile collection after each sotrastaurin dose. After the first sotrastaurin dose, consecutive amounts were 3.5 ± 8.5, 1.3 ± 2.1, and 0.6 ± 0.6 μg/day, suggesting that with improved liver function over time more tacrolimus was metabolized and less was excreted intact. At the end of the first post-transplant week, consecutive amounts excreted were 1.3 ± 2.7, 1.3 ± 2.4, and 0.6 ± 0.8 μg/day suggesting that liver metabolizing function was relatively stable with respect to tacrolimus biotransformation pathways. Given the 4–19 mg total daily doses used in these patients, the amount of tacrolimus excreted intact in bile (1–2 μg/day) was negligible and consistent with the extensive metabolism reported for this drug.

### Clinical observations

Against the complex background of clinical laboratory, vital sign, ECG and adverse event findings common to this patient population in the early post-transplant period, there was no clear signal that single-dose sotrastaurin administrations were poorly tolerated. A single serious adverse event of tacrolimus-induced disturbance of attention occurred on day 3 for which the patient was hospitalized for 2 days. Tacrolimus was replaced with ciclosporin and the patient was withdrawn from the study on day 6 completing only period 1. There were no adverse events considered by the investigators to be specifically related to sotrastaurin. Adverse events with an incidence of >20% (at least three patients reporting) from both periods combined were cholangitis (46.2%), liver transplant rejection (38.5%), urinary tract infection (23.1%), and insomnia (38.5%). Of the five treated acute rejection episodes in the first week post-transplant, four were Banff Grade I and one was Grade II.

All abnormal laboratory parameters were considered not clinically significant or not related to sotrastaurin by the study investigators. Abnormal laboratory parameters were reported as adverse events for eight patients and included: hypocalcemia, hypokalemia (two cases), hypoalbuminemia, hypophosphatemia, hyperbilirubinemia, hyperuricemia, and increased liver enzymes. All low electrolyte findings were treated and the remaining abnormalities were monitored through the end of study and did not lead to discontinuation. Clinically significant cardiac rhythm and morphology changes, reported as an adverse event, were noted for a single patient who presented with bigeminy, pathological QRS findings in V1 − V2, supraventricular extrasystole on study day 1, and onset of atrial fibrillation on study day 5. These events resolved spontaneously within 7 days of onset, were not associated with electrolyte disturbances, and were not suspected to be related to sotrastaurin. There were no clinically significant changes in rate, rhythm or conduction noted in the remaining patients' ECGs.

## Discussion

Several orally administered immunosuppressants exhibit altered pharmacokinetics early after liver transplantation.

For example, ciclosporin in the original oil-based emulsion formulation had low bioavailability in liver transplant recipients, in part, because of poor solubility in the gastrointestinal tract during external bile drainage. Solubility and bioavailability improved with a microemulsion formulation [7]. Tacrolimus blood distribution and protein binding vary significantly over time post-transplant leading to changes in free drug concentrations [8]. Mycophenolic acid apparent clearance reportedly declined 33% between week 2 and 3 after liver transplantation, with a continued slower decline thereafter to month 3 [9]. Consequently, we deemed it important to assess sotrastaurin systemic exposure after single-dose oral administration in the first week post-transplant in a limited number of *de novo* liver transplant patients before initiating larger clinical trials in this population. We were particularly interested to learn if sotrastaurin bioavailability was compromised after oral administration in patients with external bile drainage and whether the disposition of sotrastaurin changed as liver function improved and the patient recovered over the first week after surgery.

Sotrastaurin administered by mouth as single doses at the beginning and end of the first post-transplant week appeared to be adequately absorbed in liver transplant patients with external bile drainage based on total drug levels in blood. AUC's in patients were generally in the lower half of the healthy subject exposure range, although a few patients had notably lower exposure. The elimination half-life was generally in the range observed in healthy subjects or only slightly prolonged. The amount of sotrastaurin cleared in externally drained bile was negligible, but this is in line with the fact that the parent molecule is extensively biotransformed leaving little intact parent drug to excrete via this route. Among sotrastaurin metabolites, only *N*-desmethyl-sotrastaurin is known to be pharmacologically active with immunosuppressant activity similar to that of sotrastaurin. It is a minor metabolite inasmuch as its blood concentrations are <5% those of sotrastaurin in healthy subjects. The AUC of *N*-desmethyl-sotrastaurin based on total blood levels was about twofold higher in patients than in healthy subjects early in the first post-transplant week but the interpatient variability was high making it difficult to interpret the robustness of this finding. It was reassuring to observe that exposure to the metabolite tended to normalize to healthy subject levels by the end of the first week after transplantation. Based on these findings, it would appear that twice-daily dosing of sotrastaurin is appropriate for liver transplant patients. The predicted drug accumulation to steady state based on the average half-life in these patients is estimated to be 1.6-fold (close to that in healthy subjects of 1.2-fold). Pharmacokinetic data will be prospectively collected in an upcoming phase 2 trial to confirm these predictions and characterize the steady-state exposure to sotrastaurin and its active metabolite over the long-term after liver transplantation.

Sotrastaurin is primarily bound to α1-acid-glycoprotein and concentrations of this acute phase protein can be altered after liver transplantation. This raises the caveat that measurement of total drug in blood should be supplemented with measurements of free drug for a more complete understanding of drug disposition in these patients, as this is generally thought to be the pharmacologically active form that elicits a biological response. Indeed, there was a significant correlation between α1-acid-glycoprotein concentration and the fraction of sotrastaurin free in blood. When we subsequently compared free sotrastaurin exposure between patients and healthy subjects, we found that the free AUC was at the lower end of the healthy subject range or lower. While α1-acid-glycoprotein can be elevated in the first few months after liver transplantation [10], we were unable to find reports on the long-term time course of protein levels beyond month 2 after liver transplantation. We assume that α1-acid-glycoprotein levels eventually normalize which implies that free sotrastaurin concentrations would increase over time. We intend to collect longitudinal data on α1-acid-glycoprotein in an upcoming trial of sotrastaurin in *de novo* liver transplantation to learn more about temporal patterns of this acute phase protein.

The safety data collected in this patient population largely reflects the complex clinical situation in the immediate post-transplant days. We did not detect any clear signals of poor acute tolerability to single doses of sotrastaurin against this background. Clearly sotrastaurin tolerability can only be properly assessed in multicenter clinical trials over long-term observation periods.

In as much as tacrolimus can be determined in the same blood sample as sotrastaurin in the same analytical run, we took this opportunity to document tacrolimus exposure in these patients. In healthy subjects, sotrastaurin can elevate tacrolimus blood levels around twofold [11]. In renal transplant patients, this pharmacokinetic interaction was discernible but generally of a lower magnitude [12]. The tacrolimus exposures documented in this study were in the range typical in liver transplant recipients [13,14]. Given the large inter- and intrasubject variability in tacrolimus dosing and exposure in the first week post-transplant, we did not discern any clear influence of single-dose sotrastaurin on tacrolimus exposure. Whether sotrastaurin alters the pharmacokinetics of tacrolimus in liver transplant patients will need to be assessed in a long-term clinical trial including a control treatment using tacrolimus in the absence of sotrastaurin.

We excluded co-medications that are strong inhibitors or inducers of the drug metabolizing enzyme CYP3A4 because of the likelihood they could alter the pharmacokinetics of sotrastaurin, but we allowed patients to receive corticosteroids if this was routine practice at the clinical site. Corticosteroids are inducers of CYP3A4 but are generally not classified as a strong inducer. The majority of patients received prednisolone during the study with an average daily dose of 82 mg and 39 mg at the first and second pharmacokinetic visit, respectively. As only two patients did not receive prednisolone, it was not possible to make a robust comparison whether prednisolone affects the disposition of sotrastaurin. While we acknowledge that prednisolone could have an impact on sotrastaurin pharmacokinetics, nonetheless, our pharmacokinetic data constitute information on sotrastaurin exposure under clinical conditions and therefore are useful for planning further clinical trials in this patient population.

The clinical implications that we derive from this study are as follows. In general, *de novo* liver transplant recipients have normal total blood concentrations of sotrastaurin after oral administration in the first week post-transplant. Free sotrastaurin concentrations, however, may be low in some liver transplant patients, in part, because of elevated levels of α1-acid-glycoprotein. Whether sotrastaurin doses consequently need to be higher in the initial weeks after liver transplantation compared with doses used in kidney transplantation depends on the full immunosuppressive regimen in which sotrastaurin is used. Furthermore, whether therapeutic monitoring of total sotrastaurin levels in blood, determination of α1-acid-glycoprotein plasma levels, and/or occasional measurement of free sotrastaurin levels would be helpful to guide dosing in liver transplantation are issues that cannot be resolved from the data in this study alone. The optimal dosing of sotrastaurin, the other immunosuppressants with which sotrastaurin can be combined, and the need for therapeutic drug monitoring need to be addressed in future dose-finding trials. The pharmacokinetic and tolerability data from this study support the use of oral twice-daily dosing of sotrastaurin in upcoming *de novo* liver transplant clinical studies.

## Authorship

PN, UC, MW, SS, and AS: designed and performed the study; JMK, EG, KM, and AS: analyzed the data; JMK and AS: wrote the paper.

## Funding

This study was sponsored by Novartis Pharmaceuticals.

## References

1. Weckbecker G, Pally C, Beerli C, *et al.* Effects of the novel protein kinase C inhibitor AEB071 (sotrastaurin) on rat cardiac allograft survival using single agent treatment or combination therapy with cyclosporine, everolimus or FTY720. *Transpl Int* 2010; **23**: 543.
2. Budde K, Sommerer C, Becker T, *et al.* Sotrastaurin, a small molecule inhibiting protein kinase C: first clinical results in renal transplant recipients. *Am J Transplant* 2010; **10**: 571.
3. Skvara H, Dawid M, Kleyn E, *et al.* Potential therapeutic option for psoriasis with AEB071, a novel protein kinase C inhibitor. *J Clin Invest* 2008; **118**: 3151.
4. Soltes L. The Hummel-Dreyer method: impact in pharmacology. *Biomed Chromatogr* 2004; **18**: 259.
5. Banker MJ, Clark TH, Williams JA. Development and validation of a 96-well equilibrium dialysis apparatus for measuring plasma protein binding. *J Pharm Sci* 2003; **92**: 967.
6. Slade A, Vitaliti A, Agyemang A, Sieberling M, Schmouder R. AEB071: human pharmacodynamics in combination with mycophenolic acid [Abstract]. *Transplantation* 2006; **82**(Suppl. 3): 199.
7. Mueller EA, Kallay Z, Kovarik JM, *et al.* Bile-independent absorption of cyclosporine from a microemulsion formulation in liver transplant patients. *Transplantation* 1995; **60**: 515.
8. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *Ther Drug Monit* 2004; **26**: 506.
9. Bullingham RES, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 1998; **34**: 429.
10. Huang ML, Venkataramanan R, Burckhart GJ, Ptachcinski RJ, Van Thiel DH, Starzl TE. Drug-binding proteins in liver transplant patients. *J Clin Pharmacol* 1988; **28**: 505.
11. Kovarik JM, Stitah S, Slade A, *et al.* Sotrastaurin and tacrolimus coadministration: effects on pharmacokinetics and biomarker responses. *J Clin Pharmacol* 2010; **50**: 1260.
12. Kovarik JM, Steiger JU, Grinyo JM, *et al.* Tacrolimus pharmacokinetics when combined with the protein kinase C inhibitor AEB071 in *de novo* kidney transplant patients [Abstract]. *Am J Transplant* 2009; **9**(Suppl. 2): 323.
13. Florman S, Alloway R, Kalayoglu M, *et al.* Conversion of stable liver transplant recipients from a twice-daily Prograf-based regimen to a once-daily modified release tacrolimus-based regimen. *Transplant Proc* 2005; **37**: 1211.
14. Ku YM, Min DI. An abbreviated area-under-the-curve monitoring for tacrolimus in patients with liver transplants. *Ther Drug Monit* 1998; **20**: 219.