The Effects on Metabolic Clearance When Administering a Potent CYP3A Autoinducer
with the Prototypic CYP3A Inhibitor, Ketoconazole

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Running title page

Running title: Competition between a CYP3A inducer and inhibitor

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ABSTRACT

Ketoconazole is recognized as the prototypical CYP3A inhibitor, and is often used to determine the metabolic CYP3A liabilities of new chemical entities in pre-clinical and clinical studies. Ketoconazole has been commercially available for approximately 30 years and was marketed before drug-metabolizing enzymes were well characterized; consequently, little is known about its metabolism profile. Semagacestat, a gamma secretase inhibitor investigated as a potential therapy for Alzheimer’s Disease, was determined to be a potent CYP3A autoinducer in human hepatocytes. Two human studies were conducted to assess the induction potential of semagacestat. In the first study (Study 1, n=20), semagacestat increased the mean apparent clearance (CL/F) of oral midazolam (76 to 324 L/h) and nifedipine (63 to 229 L/h) as predicted from hepatocytes. In a second (steady-state) study (Study 2, n=20), semagacestat CL/F increased from 22 L/h after a single dose to 31 L/h. Ketoconazole decreased semagacestat CL/F by 32% after a single dose of semagacestat (geometric means ratio estimate [GMRE]: 0.68, 90% confidence interval [CI]: 0.64, 0.73) and 46% at steady-state (GMRE: 0.54, 90% CI: 0.51, 0.58). Ketoconazole area under the concentration-time-curve (AUC) over 8 hours decreased 49% from first to last day of semagacestat dosing. Semagacestat significantly increases the oral clearance of CYP3A substrates, confirming its inducer designation. More importantly, when administered with a potent CYP3A inducer at steady-state, ketoconazole’s plasma exposure decreased, indicating that it may also be cleared by CYP3A, other inducible enzymes or transporters, or both.
INTRODUCTION

Clinical drug-drug interaction studies are often performed during drug development to help guide clinicians in making dosing decisions when using new therapeutic agents and to confirm metabolic routes of elimination suggested by in vitro data (Wrighton and Thummel, 2000; Bjornsson et al., 2003; Fahmi and Ripp, 2010). Studies attempting to confirm the effect of metabolic inhibition are often designed as two-period crossover studies, with the experimental compound given in both periods with or without a strong inhibitor of the pathway under investigation. For the CYP3A enzymes, ketoconazole, an antifungal agent, is commonly used in these studies to examine the effect of completely inhibiting this metabolic pathway (FDA guidance, 2012).

While the effect of ketoconazole on CYP3A activity is well known, the routes of ketoconazole elimination are poorly documented in the literature. Two reports in the literature have suggested that CYP3A plays some role in ketoconazole elimination (Doble et al., 1988; Fitch et al., 2009). Nevertheless, our search of the literature revealed no clinical trial data showing the effect of a CYP3A inducer or inhibitor on the pharmacokinetics of ketoconazole. Thus, the extent to which a strong inducer or inhibitor of CYP3A metabolism may impact ketoconazole’s pharmacokinetic profile is unknown. The chemical structure of ketoconazole is presented in Figure 1a.

Semagacestat, (2S)-2-hydroxy-N-((2S)-1-((1S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-ylamino)-1-oxopropan-2-yl)-3-methylbutanamide, an azepine class γ-secretase inhibitor, was evaluated in clinical trials as a potential treatment of Alzheimer’s Disease (AD) (Olson and Albright, 2008). In humans, semagacestat has two major circulating metabolites: M2 (formed by hydrolysis of the amide bond proximal to the benzazepine ring) and M3 (formed by
benzylic hydroxylation of the benzazepine ring) (Figure 1b) (Yi et al., 2010). The formation of M3 was investigated using recombinant enzymes co-expressed with cytochrome b5 and was found to be primarily CYP3A-dependent, with the intrinsic clearance of CYP3A5 being approximately two times greater than that of CYP3A4 when catalyzing the formation of M3 (Yi et al., 2010).

In this report, we describe in vitro and clinical drug-drug interaction studies conducted during the development of semagacestat. The in vitro experiments and the first clinical study were designed to evaluate the potential for semagacestat to induce CYP3A expression. The clinical study used two different drugs to evaluate the effect of semagacestat: nifedipine and midazolam. Nifedipine, a calcium channel blocker commonly prescribed to treat hypertension, undergoes extensive metabolism in the liver (Kuroha et al., 2002) and in the intestine (Zhang et al., 2007) by CYP3A enzymes. Nifedipine was included in our study because it is a clinically relevant substrate for patients with AD and was therefore expected to be administered concomitantly with semagacestat. Midazolam is a rapid-acting benzodiazepine primarily metabolized by CYP3A (Kronbach et al., 1989) and classically used as a CYP3A substrate in exploratory settings. The second clinical study used ketoconazole to investigate the impact of CYP3A inhibition on the pharmacokinetics of semagacestat, both before and after semagacestat-induction of CYP3A. As a part of the ketoconazole-interaction study, ketoconazole concentrations were monitored both before and after induction of CYP3A by semagacestat. Together, these reports characterize the effect of semagacestat on CYP3A expression, and importantly, provide the first well-controlled clinical report of the effect of a strong CYP3A inducer on ketoconazole plasma exposure.
MATERIALS AND METHODS

In Vitro Methods

Primary Culture of Human Hepatocytes

Primary cultures of human hepatocytes were obtained from Dr. Stephen Strom, University of Pittsburgh (HH696, HH970, and HH975) (Strom et al., 1996) and from Incara Cell Technologies (Research Triangle Park, NC) (H0088) (MacDonald et al., 2001). Hepatocyte monolayers were allowed to adapt for 24 hours. Following the adaptation period, hepatocytes were incubated with semagacestat (0.1, 1, 10, or 100 μM), vehicle (0.1% dimethyl sulfoxide), or a positive control (10 μM rifampicin) in Hank’s modified medium for approximately 72 hours. Incubation medium was supplemented with 100 nM insulin, 100 nM dexamethasone, 50 μg/mL gentamicin, and 50 ng/mL amphotericin B. Each concentration of semagacestat, positive control, and vehicle control was evaluated in triplicate.

Evaluation of Testosterone 6β-Hydroxylase Activity

Testosterone metabolism to the product 6β-OH testosterone has been shown to be catalyzed by the CYP3A subfamily and was used as a form-selective catalytic activity for human CYP3A in this in vitro study. Prior to incubation with testosterone, the medium was removed and cells were pre-incubated with 3 mM salicylamide in blank HMM (testosterone samples) for 10 minutes. Salicylamide was used to prevent conjugation of the oxidative products. The cells were then incubated with 100 μM testosterone in HMM for 30 minutes. Samples of medium were collected and stored at approximately –80°C prior to analysis of the product, 6β-hydroxylase testosterone. Cells were harvested by scraping in 1.0 mL of 100 mM potassium phosphate buffer (pH 7.4), and were stored at approximately –80°C prior to determination of
protein content by the Bradford method (Bradford, 1976). The formation of TH was analyzed by high-performance liquid chromatography (Fayer et al., 2001).

**Statistical Analyses**

Single factor analysis of variance (ANOVA) was performed using Microsoft Excel 97 version SR-2 (i). Treatment and positive control groups were compared to the vehicle control. For testosterone hydroxylation activities, the samples from each of the 3 wells were analyzed by a single determination. Results were calculated as percentage of vehicle control and expressed as mean and standard error of the mean. Fold-induction results were fit to the Michaelis-Menten equation using S-Plus (Version 7.0 for Windows; Insightful Corp, Seattle, WA) to determine the maximum induction potential ($E_{\text{max}}$) and the semagacestat concentration associated with half-maximal induction ($EC_{50}$). Estimated $E_{\text{max}}$ and $EC_{50}$ values were used to predict the fold increase of CYP3A expected when semagacestat achieved a steady-state unbound concentrate ($Cu_{ss}$) using the following equation (Fahmi and Ripp, 2010):

$$R = 1 + \left(\frac{Cu_{ss}}{EC_{50} + Cu_{ss}}\right)^{E_{\text{max}}}$$

**In Vivo Methods**

Before initiation of the clinical pharmacology studies, the protocols were reviewed and approved by the National Healthcare Group Domain Specific Review Board C (Study 1; 6 Commonwealth Lane, Level 6 GMTI Building, Singapore 149547) and the Reading Independent Ethics Committee (Study 2; 93 Reading Road, Woodley, Reading, RG5 3AE, United Kingdom). The studies were conducted according to the United States Food and Drug Administration Code of Federal Regulations (21 CFR, Part 50), the Declaration of Helsinki, Good Clinical Practice...
Guidelines, and all applicable regulatory requirements. All subjects provided written informed consent before undergoing any study procedure or receiving any study treatment.

Subjects

Included in both studies were healthy men and healthy postmenopausal women, 21 to 55 (study 1) or ≥18 years old (study 2), with a body mass index of 18.5 to 29 kg/m² (study 1) or 18.5 to 32.0 kg/m² (study 2). Additionally, in study 2, individuals who used drugs that are known substrates, inducers, or inhibitors of P450 enzyme pathways or p-glycoprotein within 14 days prior to the first dose of study drug were excluded because use of these drugs might have interfered with interpretation of results regarding the effect of semagacestat.

Study Designs

Both studies were phase 1, open-label, two-period, fixed-sequence studies; the study design for each study is presented in Figure 2. During Study 1, healthy subjects received a 7.5-mg single dose of midazolam on day 1 and a 60-mg single dose of nifedipine on day 3, followed by a 5-day washout period. From day 8 through day 18 of the study, subjects received 140 mg of semagacestat once daily (QD). The 140-mg dose of semagacestat administered was the maximum dose assessed in phase 3 studies in AD patients. On day 15, subjects received a second single dose of 7.5 mg midazolam, and on day 17, they received a second single dose of 60 mg nifedipine. The doses of midazolam and nifedipine were administered at approximately the same time as semagacestat. Blood samples for pharmacokinetic analyses were collected on day 1 and day 15 approximately 0.25, 0.5, 1, 2, 3, 5, 8, 12, and 24 hours after midazolam dosing. Blood sample collections for pharmacokinetic analyses were also initiated on days 3 and 17; blood draws occurred at approximately 2, 4, 6, 8, 12, 18, 24, 28, 36, and 48 hours after nifedipine.
dosing (Figure 2a). A follow-up visit was conducted for all subjects 3 to 6 days after the last dose of semagacestat, between days 21 and 24.

Study 2 consisted of two periods, separated by a washout period of 7 to 10 days. During period 1, subjects received 140 mg semagacestat alone QD for 10 days. During period 2, subjects received 400 mg ketoconazole QD for 15 days. On days 5 to 14, subjects also received 140 mg semagacestat QD. Collection of blood samples for pharmacokinetic analyses was initiated on days 1 and 10 of period 1 and on days 5 and 14 of period 2 (Figure 2b), at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after semagacestat dosing.

For both studies, a sample size of 20 enrolled subjects with at least 16 subjects completing each study was determined to be sufficient. For study 1, the sample size determination was based upon prior observations of within-subject variability of 38% and 45% for midazolam area under the plasma concentration-time curve (AUC) and maximum plasma concentration (Cmax), respectively. The goal for study 1 was to provide a 90% confidence interval (CI) for the ratio of geometric means for midazolam’s pharmacokinetic parameters in the presence and absence of semagacestat. The CI had lower and upper confidence limits within 30% of the estimated ratio.

For study 2, based upon the prior observation of an intra-subject variability in Cmax of 20% and in AUC of 8% for semagacestat, the chosen sample size was calculated to provide a precision of approximately 10% in the natural scale for the ratio of geometric means. That is, the half-width of the 90% CI would be within 10% of the estimated ratio of means of semagacestat + ketoconazole compared with semagacestat alone.
Bioanalytical Analyses

Plasma samples were analyzed for midazolam, nifedipine, and semagacestat (study 1) or semagacestat, its metabolites M2 and M3, and ketoconazole (study 2) using validated liquid chromatography with tandem mass spectroscopy (LC/MS/MS) methods. Samples were analyzed by Prevalere Life Sciences, Inc. (Whitesboro, New York). The quantification ranges were 0.500 to 100 ng/mL for midazolam, 1.00 to 100 ng/mL for nifedipine, 0.200 to 250 ng/mL for semagacestat, M2, and M3, and 5.00 to 1000 ng/mL for ketoconazole. Inter-assay accuracy (presented as percentage relative error) was ≤12.1% for midazolam, ≤9.7% for nifedipine, ≤6.3% for semagacestat, ≤0.9% for M2, ≤4.8% for M3, and ≤9.2% for ketoconazole. The inter-assay precision (presented as percentage relative standard deviation) was ≤5.7% for midazolam, ≤5.4% for nifedipine, ≤7.7% for semagacestat, ≤10.8% for M2, ≤14.3% for M3, and ≤9.0% for ketoconazole.

Pharmacokinetic Analyses

Pharmacokinetic parameters were computed with standard noncompartmental methods of analysis using WinNonlin Enterprise 5.0.1 (Pharsight; Sunnyvale, CA). The following pharmacokinetic parameters were determined: $C_{\text{max}}$, time to maximum plasma concentration ($t_{\text{max}}$), $\text{AUC}$ (AUC$_\text{t}$ for the M2 metabolite due to its long half-life), apparent clearance ($\text{CL/F}$), apparent volume of distribution ($\text{Vz/F}$), and terminal half-life ($t_{1/2}$). Actual sampling times were used in the analyses. Values below the quantifiable limit were treated as missing data.

Safety

In both studies, safety assessments included the following measurements at regular intervals throughout the studies: supine blood pressure and pulse rate, electrocardiograms, clinical laboratories, and solicited and unsolicited adverse events.
Statistical Analyses

For both studies, pharmacokinetic parameters were log-transformed and analyzed using a linear mixed-effects model with treatment as the fixed effect and subject as the random effect. During study 1, the ratios of the geometric least-squares (LS) means and the corresponding 90% CIs of midazolam and nifedipine were evaluated in the presence and absence of semagacestat. Primary pharmacokinetic parameters for midazolam and nifedipine were \( \text{AUC}(0-\infty), C_{\text{max}}, \) and \( t_{\text{max}} \); additionally, \( \text{CL/F} \) and \( \text{AUC}(0-\text{tlast}) \) were calculated. During study 2, \( C_{\text{max}}, \) \( \text{AUC}, \) and \( \text{CL/F} \) of a single dose of semagacestat (day 1 in period 1 and day 5 in period 2) were analyzed using the described mixed-effect model. The ratios of geometric LS means and 90% CIs between semagacestat + ketoconazole to semagacestat alone were calculated (day 5 in period 2 vs. day 1 in period 1). Similarly, multiple doses of semagacestat and their metabolites (M2 and M3) were analyzed. In addition, the effect of multiple doses of semagacestat on \( \text{CL/F} \) was evaluated comparing day 14 of period 2 to day 5 of period 2. The \( C_{\text{max}} \) and \( \text{AUC} \) values on days 0 to 8 for ketoconazole were determined at steady-state when coadministered with placebo or semagacestat.

RESULTS

Preclinical Studies

CYP3A Induction by Semagacestat

Following 3 days of treatment with semagacestat (0.1, 1, 10, and 100 µM), a dose-dependent increase in CYP3A-mediated TH activity was observed in all 4 of the hepatocyte preparations (HH969, HH970, HH975, and H0088) examined (Figure 3). Table 1 presents rates of testosterone 6β-hydroxylation in four human hepatocyte preparations and fold induction relative to the vehicle and positive control (rifampin 10 µM). The mean testosterone TH activity
for each treatment group demonstrates that the baseline and rifampin-induced activities are similar to those previously reported (Kostrubsky et al., 1999). The increase in CYP3A-mediated TH activity was statistically significant for all hepatocyte preparations treated with 1 μM semagacestat and higher ($p<.05$). In incubations containing 10 μM semagacestat, a significant increase in CYP3A-mediated TH activity was reported across all hepatocyte preparations in a range of approximately 4- to 12-fold when compared to activity in vehiclecontrol incubations (Figure 3) ($p<.05$). Each preparation also showed a significant induction of CYP3A-mediated TH activity with the positive control rifampicin (10 μM), yielding 14.3-, 6.9-, 3.6-, and 9.9-fold increases in preparations HH969, HH970, HH975, and H0088, respectively. The mean fold change relative to vehicle and positive control is shown in Table 1. Therefore, in cultures of human hepatocytes, semagacestat at 10 and 100 μM produced a CYP3A induction response equal to that of the positive control, rifampicin. The value of $E_{max}$ for semagacestat was estimated to be 11.6-fold (standard error of the estimate [SEE]: 0.5), while $EC_{50}$ was estimated to be 0.81 μM (SEE: 0.16). These values along with the average unboundsteady-state concentration of semagacestat after daily dosing of 140 mg of approximately 0.37 μM (fraction unbound in plasma is 0.7) predict a 4.6-fold increase in hepatic CYP3A content (Fahmi et al., 2009).

Clinical Pharmacology Studies

Subject Baseline Demographics and Disposition

Both studies together had 40 subjects. Baseline demographic characteristics of subjects in each study are presented in Table 2. In study 1, 18 subjects completed the study (of those, 3 subjects did not receive their last dose of semagacestat due to rash) and 2 subjects discontinued; in study 2, 18 subjects completed the study and 2 subjects discontinued.
Effect of CYP3A Induction by Semagacestat on Midazolam and Nifedipine Pharmacokinetics

(Study 1 Results)

Coadministration of 140 mg semagacestat for 10 days with either a single 7.5-mg dose of midazolam or a single 60-mg dose of nifedipine resulted in diminished plasma concentrations of both midazolam and nifedipine compared with plasma concentrations during midazolam- or nifedipine-only administration (Table 3 and Supplemental Figures 1a and 1b). During coadministration with semagacestat, the oral clearance of midazolam increased approximately 4.25-fold, while the oral clearance of nifedipine increased approximately 3.65-fold (Table 2).

Effect of CYP3A4 Inhibition by Ketoconazole on Semagacestat Pharmacokinetics (Study 2 Results)

Plasma concentrations of semagacestat increased following both a single 140-mg dose and multiple 140-mg doses for 10 days when coadministered with 400 mg ketoconazole administered for 15 days (Supplemental Figures 2a and 2b). Compared with semagacestat alone, oral clearance of semagacestat was reduced 32% following a single dose and 46% following multiple doses when coadministered with ketoconazole (Table 4). After multiple doses of semagacestat alone, a higher semagacestat plasma clearance (31.3 L/h) was observed compared with a single dose of semagacestat alone (22.1 L/h), indicative of autoinduction (Table 3).

The AUC of semagacestat’s M3 metabolite (formed by CYP3A) increased following autoinduction during semagacestat-only administration and decreased during coadministration with ketoconazole (Table 4 and Supplemental Figure 3a). On the contrary, the AUC of semagacestat decreased with autoinduction, while the AUCs of both semagacestat and its M2 metabolite (not formed by CYP3A) increased during coadministration with ketoconazole.
(Table 4 and Supplemental Figure 3b). The AUC of the M2 metabolite was essentially unchanged after autoinduction by semagacestat (Table 4 and Supplemental Figure 3b).

After multiple doses of semagacestat, the AUC from time zero to 8 hours (AUC(0-8)) of ketoconazole was reduced by approximately 50% (Table 5 and Supplemental Figure 4).

Safety (Studies 1 and 2)

One serious adverse event was observed, during which a subject experienced an anaphylactic reaction 5 minutes after first co-administration of semagacestat and ketoconazole; this subject was discontinued from study 2. A total of 3 other subjects discontinued from studies 1 and 2 combined because of (nonserious) adverse events: asymptomatic elevated lipase levels after 10 days of semagacestat dosing; rash after 9 daily doses of semagacestat; and elevated liver enzymes 11 days after the last of 10 daily doses of semagacestat. All subjects recovered without clinical intervention and without sequela. Except for the above noted events, no clinically significant changes in vital signs and electrocardiograms occurred in the course of either study.

The most frequently observed treatment-emergent adverse events in both studies in subjects who received semagacestat alone or in combination with other study drugs were rash and pruritus. A total of 8 subjects developed rash (semagacestat+midazolam, n=3; semagacestat+nifedipine, n=2; semagacestat, n=2; semagacestat+ketoconazole, n=1) and 4 subjects experienced pruritus (semagacestat+midazolam, n=1; semagacestat+nifedipine, n=1; semagacestat, n=1; semagacestat+ketoconazole, n=1).

DISCUSSION

Ketoconazole has been recognized as the prototypical CYP3A inhibitor and is used to determine the fraction of the dose cleared by CYP3A for new chemical entities both pre-
clinically and clinically. Approximately 50% of drugs that are eliminated by metabolism rely upon CYP3A enzymes to some degree and there are numerous clinically important inhibitors and inducers of CYP3A4 and CYP3A5 (Bennett, 1995). Semagacestat, recently suspended for further development as a treatment for AD, is in part metabolized by CYP3A enzymes, and therefore, this series of studies was designed to quantify the potential for CYP3A enzymes to be inhibited or induced by semagacestat and to define the effect of CYP3A inhibition on semagacestat exposure.

Radiolabeled mass balance studies revealed that following a single dose, semagacestat has good oral absorption (≥87%) and approximately 44% of the total dose is excreted unchanged in urine, 20% undergoes amide hydrolysis to M2, and the remaining 20% undergoes oxidation to M3 by CYP3A4 and CYP3A5 (Yi et al., 2010). Thus, approximately 23% of the absorbed dose is oxidized by CYP3A4 and CYP3A5. Semagacestat was not predicted to be an inhibitor of any CYP enzyme or any transporter, based on in vitro experiments with human liver microsomes and transporter expression systems. However, when semagacestat was evaluated as an inducer of CYP3A4 in primary cultures of human hepatocytes, it displayed a maximal induction extent and potency comparable to the prototypical strong CYP3A4 inducer rifampicin (Figure 1). The induction parameters estimated from this in vitro study predict that a substantial 4.6-fold induction of hepatic CYP3A4 protein would be expected at the unbound drug concentrations expected at steady-state following dosing with the clinically-tested 140-mg daily dose. The approach we used to predict the in vivo increase in hepatic CYP3A4 due to an inducer has been previously demonstrated to perform well over a range of established CYP3A4 inducers (Fahmi et al., 2009).
In view of the significant increase in hepatic CYP3A4 content predicted from our human hepatocyte studies, we chose to conduct a clinical study to evaluate the effect of steady-state semagacestat concentrations on the oral clearance of two well-established and clinically important CYP3A substrates, midazolam and nifedipine. As expected, the oral clearances of midazolam and nifedipine were significantly increased by 4.2- and 3.6-fold, respectively (Table 2). One effect of semagacestat was to reduce the $C_{\text{max}}$ of midazolam and nifedipine, suggesting that the bioavailability of these drugs was reduced. This observation is consistent with the abundant expression of CYP3A4 in the gut wall and the capability of CYP3A inducers to up-regulate this expression (Gorski et al., 1998). A second feature of the semagacestat effect was a greater increase in oral clearance than could be accounted for by the change in bioavailability suggested by the decrease in $C_{\text{max}}$ of midazolam and nifedipine (Table 2). This reflects an increase in systemic clearance and would be expected given that the systemic clearance reflects the CYP3A activity in the liver.

Overall, the extent of CYP3A induction caused by the 140-mg daily dose of semagacestat is less than that associated with rifampicin induction, despite similarity in the in vitro EC$_{50}$ and E$_{\text{max}}$ in hepatocytes, because the steady-state unbound concentrations are not at the E$_{\text{max}}$ of the induction response. If higher doses of semagacestat were explored to optimize therapeutic response, a greater extent of CYP3A induction could be expected. The prototypical CYP3A4 inducer, rifampin, reaches unbound plasma concentrations that cause maximal induction at therapeutic doses and reduces midazolam oral AUC to 0.05 times the baseline value, and we expect semagacestat to elicit a similar effect at sufficiently high plasma exposures (Gorski et al., 2003). Although the mechanism of CYP3A up-regulation has not been addressed in this study, it is reasonable to assume that activation of the nuclear factor pregnane X receptor (PXR) is
involved. A potential up-regulation of PXR is a significant observation, because a number of other CYPs, such as CYP2C9 and CYP2B6, as well as some transporters, such as p-glycoprotein and OATP1B1, are also up-regulated when PXR is activated (Urquhart et al., 2007).

Semagacestat is not only an inducer of CYP3A enzymes but is also partly metabolized by these enzymes, and as expected, semagacestat was found to be an autoinducer. The oral clearance of semagacestat increased from 22.1 L/h after a single dose to 31.3 L/h after multiple daily dosing (Table 3). This observation is consistent with high bioavailability and a modest contribution, approximately 23%, of CYP3A to the overall semagacestat CL/F, and of a 4- to 5-fold increase in CYP3A activity in response to multiple doses of semagacestat. The formation of the M3 metabolite of semagacestat is catalyzed by CYP3A4 and CYP3A5, and as expected, the AUC of this metabolite increased by approximately 50% following autoinduction (Table 3); exposure to M2, a non-CYP3A metabolite, was unchanged between single and multiple dosing with semagacestat (Yi et al 2010).

To determine the sensitivity of semagacestat to inhibition of CYP3A by a strong in vivo inhibitor, we examined the effect of ketoconazole on the oral clearance and exposure of semagacestat after single- and multiple-dose treatments. This relatively complex drug-drug-interaction study design was necessary because the contribution of CYP3A enzymes to semagacestat clearance was expected to increase upon multiple dosing. As expected from the proposed 23% contribution of CYP3A to the clearance of semagacestat, ketoconazole reduced the single dose oral clearance from 22.1 L/h to 15.1 L/h, suggesting that CYP3A was responsible for 32% of the oral clearance of semagacestat after a single dose. A much greater relative reduction from 31.3 to 17.0 L/h was noted after multiple dosing of semagacestat and ketoconazole (Table 3). These data are consistent with an autoinduction phenomenon increasing
the CYP3A contribution to semagacestat clearance to approximately 46%. Exposure to the CYP3A-formed metabolite, M3, was reduced substantially by ketoconazole following single and multiple dosing with semagacestat, but due to the increased AUC of parent drug, exposure to the non-CYP3A metabolite, M2, was increased (Table 3).

This single-dose/multiple-dose study design allows prescribers to judge the impact of CYP3A inhibition on the victim drug exposure under two conditions of interest; first, when semagacestat is added to a stable dosing regimen of inhibitor, and second, when an inhibitor is added to a stable dosing regimen of semagacestat. Although the fold changes in semagacestat exposure caused by ketoconazole differ between single and multiple doses, the residual AUC of semagacestat is essentially unaffected because the inhibitor reduces oral clearance to the same non-CYP3A value in both scenarios.

Ketoconazole exposure was markedly decreased following coadministration with semagacestat (Table 4). The mechanism of ketoconazole clearance is poorly documented in the literature; however, there is evidence to suggest that it may be at least partly metabolized by CYP3A (Doble et al., 1988; Fitch et al., 2009). While the results of this study appear to support that CYP3A is a significant clearance pathway for ketoconazole, it cannot be discounted that semagacestat may also induce other CYP enzymes or transporters via PXR or constitutive active/androstane receptor pathways, which may account for potential alternative clearance mechanisms for ketoconazole. Although the change in ketoconazole exposure appears to be significant, its impact on the results of this study appears to be minimal. It is noted that there was a small (approximately 13%), though statistically significant, increase in semagacestat clearance after multiple doses were coadministered with ketoconazole. While it is possible that this increase in semagacestat clearance after multiple doses coadministered with ketoconazole is
caused by the induction of an alternative, minor clearance pathway that is not inhibited by ketoconazole, the effect is more likely due to a change in ketoconazole exposure (i.e., lower ketoconazole plasma levels as described above). This emphasizes the importance of monitoring ketoconazole exposure in drug-drug-interaction studies with compounds that may induce or inhibit CYP activity to avoid insufficient inhibition of CYP3A activity or inadvertent excessive exposure to ketoconazole which might lead to toxicity. However, in this study, subjects were given ketoconazole over 2 weeks, and no adverse events indicative of enhanced ketoconazole toxicity during long-term exposure were observed.

A total of four subjects (two in each study) were discontinued from the studies because of treatment-emergent adverse events. In three instances, those events (i.e., rash, lipase enzyme elevation, and elevated liver enzymes) occurred after multiple doses of semagacestat. It is unclear if autoinduction of semagacestat contributed to these events. The fourth discontinuation occurred as the result of an anaphylactic shock on day 1 of coadministration of semagacestat with ketoconazole. In both clinical studies, semagacestat was generally well-tolerated when administered alone or in combination with midazolam, nifedipine, or ketoconazole.

In conclusion, the data from the preclinical and clinical pharmacology studies combined demonstrated that semagacestat is a moderate to strong inducer, autoinducer, and substrate of CYP3A. Results suggest that coadministering ketoconazole with a potent CYP3A inducer may reduce the exposure to ketoconazole, which may have an impact when studying new chemical entities that are known to be inducers. Additionally, there are implications for clinical practice; if ketoconazole is added to an already steady-state CYP3A inducer, an adjusted dose of both compounds may be required.
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Conducted experiments: de la Peña, Wise
Contributed new reagents or analytic tools: Ayan-Oshodi
Performed data analysis: Annes, Ayan-Oshodi, Brown, Friedrich, Hall, Lowe, Willis, Zhang,
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REFERENCES


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d) Financial interest disclosures: Ms. Ayan-Oshodi, Mr. Brown, and Drs. Willis, Annes, Lowe, Friedrich, de la Peña, Zhang, Wise, and Hall are full-time employees and minor stockholders of Eli Lilly and Company and its subsidiaries.
FIGURE LEGEND

Figure 1. Chemical structures.
   1a) Ketoconazole
   1b) Metabolic fate of semagacestat

Figure 2. Study designs for study 1 (a) and study 2 (b).

Figure 3. In vitro induction of CYP3A4 activity by semagacestat in primary cultures of human hepatocytes.

Abbreviations: Prep=preparation; SMG=semagacestat.

Presented are means ± standard error (n=3 per donor).

* p-value <0.05. Treatment and positive control groups were compared to the vehicle control.

"Samples found to have microbial contamination – no data available."
Table 1: Mean (standard deviation) testosterone 6β-hydroxylase activity in four human hepatocyte preparations and fold induction relative to the vehicle and positive control (rifampin 10 µM)

<table>
<thead>
<tr>
<th>Activity (pmol/min/mg)</th>
<th>Fold Induction Over Positive Control</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Vehicle Control</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4</td>
<td>152</td>
<td>66</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td>Rifampin 10 µM (n=4)</td>
<td></td>
<td>4</td>
<td>1102</td>
<td>238</td>
<td>7.3</td>
<td>1</td>
</tr>
<tr>
<td>Semagacestat 0.1 µM</td>
<td></td>
<td>4</td>
<td>190</td>
<td>81</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Semagacestat 1 µM</td>
<td></td>
<td>4</td>
<td>625</td>
<td>94</td>
<td>4.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Semagacestat 10 µM</td>
<td></td>
<td>4</td>
<td>1049</td>
<td>144</td>
<td>6.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Semagacestat 100 µM</td>
<td></td>
<td>3</td>
<td>1192</td>
<td>42</td>
<td>7.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Abbreviations: SD=standard deviation
Table 2. Baseline Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study 1 (N=20)</th>
<th>Study 2 (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (range)</td>
<td>30.0 (21-46)</td>
<td>40.8 (22-74)</td>
</tr>
<tr>
<td>Gender, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Race, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Asian</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Mean body weight, kg (range)</td>
<td>71.9 (61.6–88.6)</td>
<td>81.2 (65.7-102.7)</td>
</tr>
<tr>
<td>Mean BMI, kg/m$^2$ (range)</td>
<td>23.7 (19.7-27.1)</td>
<td>26.4 (20.4-31.8)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI=body mass index; N=number of subjects; n=number of affected subjects.
Table 3. Statistical Comparison of Midazolam and Nifedipine Pharmacokinetics in the Presence and Absence of Semagacestat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>N</th>
<th>LS GeoMean (90% CI)</th>
<th>GMRE (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Midazolam</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC((0-\infty), \text{ng\cdot h/mL})</td>
<td>MDZ only</td>
<td>20</td>
<td>98.3 (86.1, 112)</td>
<td>0.24 (0.20, 0.28)</td>
</tr>
<tr>
<td></td>
<td>MDZ + SMG</td>
<td>20</td>
<td>23.2 (20.3, 26.5)</td>
<td></td>
</tr>
<tr>
<td>AUC((0-t_{\text{last}}), \text{ng\cdot h/mL})</td>
<td>MDZ only</td>
<td>20</td>
<td>93.1 (81.1, 107)</td>
<td>0.23 (0.19, 0.27)</td>
</tr>
<tr>
<td></td>
<td>MDZ + SMG</td>
<td>20</td>
<td>21.4 (18.7, 24.6)</td>
<td></td>
</tr>
<tr>
<td>C_{\text{max}}, \text{ng/mL}</td>
<td>MDZ only</td>
<td>20</td>
<td>37.8 (31.5, 45.4)</td>
<td>0.37 (0.29, 0.47)</td>
</tr>
<tr>
<td></td>
<td>MDZ + SMG</td>
<td>20</td>
<td>14.0 (11.7, 16.8)</td>
<td></td>
</tr>
<tr>
<td>Median t_{\text{max}}, h</td>
<td>MDZ only</td>
<td>20</td>
<td>0.5 (0.3, 2.0)</td>
<td>NA</td>
</tr>
<tr>
<td>(range)</td>
<td>MDZ + SMG</td>
<td>20</td>
<td>0.5 (0.3, 1.0)</td>
<td></td>
</tr>
<tr>
<td>CL/F, L/h (CV%)</td>
<td>MDZ only</td>
<td>20</td>
<td>76.3 (28.9)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>MDZ + SMG</td>
<td>20</td>
<td>324 (42.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Nifedipine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC((0-\infty), \text{ng\cdot h/mL})</td>
<td>NFP only</td>
<td>18</td>
<td>948 (757, 1186)</td>
<td>0.32 (0.24, 0.43)</td>
</tr>
<tr>
<td></td>
<td>NFP + SMG</td>
<td>15</td>
<td>304 (243, 380)</td>
<td></td>
</tr>
<tr>
<td>AUC((0-t_{\text{last}}), \text{ng\cdot h/mL})</td>
<td>NFP only</td>
<td>20</td>
<td>866 (746, 1006)</td>
<td>0.27 (0.22, 0.33)</td>
</tr>
<tr>
<td></td>
<td>NFP + SMG</td>
<td>19</td>
<td>236 (202, 275)</td>
<td></td>
</tr>
<tr>
<td>C_{\text{max}}, \text{ng/mL}</td>
<td>NFP only</td>
<td>20</td>
<td>40.9 (36.5, 45.7)</td>
<td>0.23 (0.20, 0.27)</td>
</tr>
<tr>
<td></td>
<td>NFP + SMG</td>
<td>19</td>
<td>9.54 (8.5, 10.7)</td>
<td></td>
</tr>
<tr>
<td>Median t_{\text{max}}, h</td>
<td>NFP only</td>
<td>20</td>
<td>18.0 (6.0, 36.0)</td>
<td>NA</td>
</tr>
<tr>
<td>(range)</td>
<td>NFP + SMG</td>
<td>19</td>
<td>18.0 (6.2, 48.0)</td>
<td></td>
</tr>
<tr>
<td>CL/F, L/h (CV%)</td>
<td>NFP only</td>
<td>18</td>
<td>62.8 (52.7)(^a)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>NFP + SMG</td>
<td>15</td>
<td>229 (30.8)(^b)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AUC\((0-\infty)=area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC\((0-t_{\text{last}})=area under the plasma concentration-time curve from time zero until the time of the last measurable concentration; CI=confidence interval; CL/F=apparent clearance; C_{\text{max}}=maximum plasma concentration; CV=coefficient of variation;
GMRE=geometric means ratio estimate; LS GeoMean= east-squares geometric mean;
MDZ=midazolam; N=number of subjects; NA=not available; NFP=nifedipine;
SMG=semagacestat; $t_{\text{max}}$=time to maximum plasma concentration.

The data are geometric means (CV%) unless indicated differently.

$^a$Terminal slope could not be accurately determined in 2 subjects.

$^b$Terminal slope could not be accurately determined in 4 subjects.
Table 4. Effect of Ketoconazole on the Pharmacokinetics of Semagacestat and Its Metabolites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>N</th>
<th>LS GeoMean (90% CI)</th>
<th>GMRE (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single-dose SMG – Effect on SMG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0-\infty}), ng·h/mL</td>
<td>SMG(^a)</td>
<td>20</td>
<td>6320 (5740, 6970)</td>
<td>1.46 (1.38, 1.56)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^b)</td>
<td>18</td>
<td>9260 (8400, 10200)</td>
<td></td>
</tr>
<tr>
<td>C(_{\text{max}}), ng/mL</td>
<td>SMG(^a)</td>
<td>20</td>
<td>1640 (1420, 1900)</td>
<td>1.25 (1.06, 1.46)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^b)</td>
<td>18</td>
<td>2050 (1760, 2390)</td>
<td></td>
</tr>
<tr>
<td>CL/F, L/h</td>
<td>SMG(^a)</td>
<td>20</td>
<td>22.1 (20.1, 24.4)</td>
<td>0.68 (0.64, 0.73)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^b)</td>
<td>18</td>
<td>15.1 (13.7, 16.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple-dose SMG – Effect on SMG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{t,ss}), ng·h/mL</td>
<td>SMG(^c)</td>
<td>20</td>
<td>4480 (4140, 4850)</td>
<td>1.84 (1.72, 1.97)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^d)</td>
<td>18</td>
<td>8230 (7580, 8930)</td>
<td></td>
</tr>
<tr>
<td>C(_{\text{max,ss}}), ng/mL</td>
<td>SMG(^c)</td>
<td>20</td>
<td>1280 (1130, 1440)</td>
<td>1.46 (1.26, 1.70)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^d)</td>
<td>18</td>
<td>1860 (1640, 2120)</td>
<td></td>
</tr>
<tr>
<td>CL(_{ss}/F), L/h</td>
<td>SMG(^c)</td>
<td>20</td>
<td>31.3 (28.9, 33.9)</td>
<td>0.54 (0.51, 0.58)</td>
</tr>
<tr>
<td></td>
<td>SMG + KTZ(^d)</td>
<td>18</td>
<td>17.0 (15.7, 18.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Effect on Metabolite M2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-dose SMG</td>
<td>SMG(^a)</td>
<td>20</td>
<td>1800 (1650, 1960)</td>
<td>1.29 (1.20, 1.37)</td>
</tr>
<tr>
<td>AUC(_{0-\infty}), ng·h/mL</td>
<td>SMG + KTZ(^b)</td>
<td>18</td>
<td>2320 (2120, 2530)</td>
<td></td>
</tr>
<tr>
<td>Multiple dose SMG</td>
<td>SMG(^c)</td>
<td>20</td>
<td>1890 (1740, 2050)</td>
<td>1.32 (1.22, 1.43)</td>
</tr>
<tr>
<td>AUC(_{t,ss}), ng·h/mL</td>
<td>SMG + KTZ(^d)</td>
<td>18</td>
<td>2490 (2290, 2710)</td>
<td></td>
</tr>
<tr>
<td><strong>Effect on Metabolite M3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-dose SMG</td>
<td>SMG(^a)</td>
<td>20</td>
<td>1060 (878, 1290)</td>
<td>0.23 (0.19, 0.29)</td>
</tr>
<tr>
<td>AUC(_t), ng·h/mL</td>
<td>SMG + KTZ(^b)</td>
<td>18</td>
<td>246 (201, 302)</td>
<td></td>
</tr>
<tr>
<td>Multiple dose SMG</td>
<td>SMG(^c)</td>
<td>20</td>
<td>1590 (1350, 1860)</td>
<td>0.35 (0.30, 0.42)</td>
</tr>
<tr>
<td>AUC(_{t,ss}), ng·h/mL</td>
<td>SMG + KTZ(^d)</td>
<td>18</td>
<td>560 (474, 663)</td>
<td></td>
</tr>
</tbody>
</table>
Abbreviations: AUC(0-\(\infty\))=area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC\(_T\)=area under the concentration versus time curve during 1 dosing interval; AUC\(_{T,ss}\)=area under the concentration versus time curve during 1 dosing interval at steady-state; CI=confidence interval; CL/F=apparent clearance; CL\(_{ss}/F\)=apparent total clearance at steady-state; \(C_{max}\)=maximum observed plasma concentration; \(C_{max,ss}\)=maximum observed plasma concentration at steady-state; GMRE=geometric means ratio estimate;
KTZ=ketoconazole; LS GeoMean=least-squares geometric mean; N=number of subjects;
SMG=semagacestat.

\(^a\)SMG single dose alone (140 mg), day 1 of period 1.

\(^b\)SMG single dose (140 mg) + KTZ once daily (400 mg), day 5 of period 2.

\(^c\)SMG once daily alone (140 mg), day 10 of period 1.

\(^d\)SMG once daily (140 mg) + KTZ once daily (400 mg), day 14 of period 2.

Note: Tmax for semagacestat was 1 hr across periods (range: 0.5 – 4.0 hr).
Table 5. Pharmacokinetics of Ketoconazole Following Single/Multiple Doses of Semagacestat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Geometric Mean (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SMG SD + KTZ QD&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, ng/mL</td>
<td>4980 (259)</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;, &lt;sup&gt;c&lt;/sup&gt; h (range)</td>
<td>2.00 (0.92, 4.00)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-8)&lt;/sub&gt;, ng·h/mL</td>
<td>25100 (304)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC<sub>(0-8)</sub>=area under the concentration versus time curve from time zero to 8 hours; C<sub>max</sub>=maximum observed drug concentration; CV=coefficient of variation; KTZ=ketoconazole (400 mg); N=number of subjects; QD=once daily; SMG=semagacestat (140 mg); SD=single dose; t<sub>max</sub>=time of C<sub>max</sub>.

<sup>a</sup>Day 5 of period 2.

<sup>b</sup>Day 14 of period 2.

<sup>c</sup>Median (range).
Figure 1b
Figure 2a

SMG = Semagacestat
MDZ = Midazolam
NFP = Nifedipine
PK = Pharmacokinetics

SMG Trough and Peak PK on Day 15
Figure 2b
Figure 3
SUPPLEMENTAL MATERIAL

The Effects on Metabolic Clearance When Administering a Potent CYP3A Autoinducer with the Prototypic CYP3A Inhibitor, Ketoconazole

Mosun A. Ayan-Oshodi, MS; Brian A. Willis, PhD; William F. Annes, BS; Stephen L. Lowe, PhD; Stuart Friedrich, PhD; Amparo de la Peña, PhD; Wei Zhang, PhD; Thomas Brown, MS; Stephen D. Wise, MD; Stephen D. Hall, PhD

Drug Metabolism and Disposition
Figure Legend:

Supplemental Figure 1a. Pharmacokinetic profile of midazolam.

Supplemental Figure 1b. Pharmacokinetic profile of nifedipine.

Supplemental Figure 2a. Pharmacokinetic profile of semagacestat after single dose.

Supplemental Figure 2b. Pharmacokinetic profile of semagacestat after multiple doses.

Supplemental Figure 3a. Pharmacokinetic profile of M3 formed by CYP3A.

Supplemental Figure 3b. Pharmacokinetic profile of M2 formed by hydrolysis.

Supplemental Figure 4. Pharmacokinetic profile of ketoconazole.
Supplemental Figure 1a.

Abbreviations: SMG=semagacestat (140 mg).

Midazolam (7.5 mg)

Presented are means ± standard deviations.
Supplemental Figure 1b.

Abbreviations: SMG=semagacestat (140 mg).

Nifedipine (60 mg).

Presented are means ± standard deviations.
Supplemental Figure 2a.

Abbreviations: KTZ=ketoconazole (400 mg); QD=once daily; SMG=semagacestat (140 mg).

Presented are means ± standard deviations.
Supplemental Figure 2b.

Abbreviations: KTZ=ketoconazole (400 mg); QD=once daily; SMG=semagacestat (140 mg).

Presented are means ± standard deviations.
Abbreviations: KTZ=ketoconazole (400 mg); QD=once daily; SMG=semagacestat (140 mg).

Presented are means ± standard deviations.
Supplemental Figure 3b.

Abbreviations: KTZ=ketoconazole (400 mg); QD=once daily; SMG=semagacestat (140 mg).

Presented are means ± standard deviations.
Supplemental Figure 4.

![Graph showing the plasma concentration of ketoconazole over time for SMG QD + KTZ QD (Period 2, Day 5) and SMG QD + KTZ QD (Period 2, Day 14).](image-url)