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


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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 preclinical 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 metabolic profile. Semagacestat, a γ-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 (76–324 l/h) and nifedipine (63–229 l/h) as predicted from hepatocytes. In a second (steady-state) study (study 2, n = 20), semagacestat CL/F increased from 22 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, 0.68; 90% confidence interval (CI), 0.64, 0.73] and 46% at steady state [geometric means ratio estimate, 0.54; 90% CI, 0.51, 0.58]. Ketoconazole area under the concentration-time curve over 8 h 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 (U.S. Food and Drug Administration, http://www.fda.gov/downloads/%20Drugs/GuidanceCompliance RegulatoryInformation/Guidances/UCM292362.pdf, 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 affect ketoconazole’s pharmacokinetic profile is unknown. The chemical structure of ketoconazole is presented in Fig. 1A.

Semagacestat [(2S)-2-hydroxy-N-[(2S)-1-[(1S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzof[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

ABBREVIATIONS: AD, Alzheimer’s disease; HMM, Hanks’ modified medium; P450, cytochrome P450; AUC, area under the plasma concentration-time curve; CI, confidence interval; CL/F, apparent clearance; Emax, maximal induction potential; PXR, pregnane X receptor; TH, 6β-hydroxylase testosterone.
bond proximal to the benzazepine ring) and M3 (formed by benzylic hydroxylation of the benzazepine ring) (Fig. 1B) (Yi et al., 2010). The formation of M3 was investigated using recombinant enzymes coexpressed with cytochrome b5 and was found to be primarily CYP3A-dependent, with the intrinsic clearance of CYP3A5 being approximately 2 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. Taken together, these reports characterize the effect of semagacestat on CYP3A expression and 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 (HHH96, HHH970, and HHH975) (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 h. After the adaptation period, hepatocytes were incubated with semagacestat (0.1, 1, 10, or 100 nM), vehicle (0.1% dimethyl sulfoxide), or a positive control (10 nM rifampicin) in Hanks’ modified medium (HMM) for approximately 72 h. 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-hydroxylase testosterone (TH) 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. Before incubation with testosterone, the medium was removed and cells were preincubated with 3 nM
salicylamide in blank HMM (testosterone samples) for 10 min. Salicylamide was used to prevent conjugation of the oxidative products. The cells were then incubated with 100 μM testosterone in HMM for 30 min. Samples of medium were collected and stored at approximately −80°C before 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 before 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).

### Results

**Pharmacokinetic 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 spectrometry methods. Samples were analyzed by Prevarelle Life Sciences Inc. (Whitesboro, New York). The quantification ranges were 0.500 to 100 for midazolam, 1.00 to 100 for nifedipine, 0.200 to 250 for semagacestat, M2, and M3, and 5.00 to 1000 ng/ml for ketoconazole. Interassay 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 interassay precision (presented as percentage relative S.D.) was ±7.5% 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, Mountain View, CA). The following pharmacokinetic parameters were determined: Cmax, time to maximum plasma concentration (tmax), AUC (AUC, for the M2 metabolite because of its long t1/2), apparent clearance (CL/F), apparent volume of distribution (V/F), and terminal t1/2. 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 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 AUC from time zero to infinity, Cmax, and tmax. In addition, CL/F and AUC from time zero until the time of the last measurable concentration were calculated. During study 2, Cmax, AUC, and 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 least-squares means and 90% CIs between semagacestat plus ketoconazole to semagacestat alone were calculated (day 5 in period 2 versus day 1 in period 1). Likewise, multiple doses of semagacestat and their metabolites (M2 and M3) were analyzed. In addition, the effect of multiple doses of semagacestat on CL/F was evaluated comparing day 14 of period 2 with day 5 of period 2. The Cmax and AUC values from 0 to 8 h after dosing for ketoconazole were determined at steady state when coadministered with placebo or semagacestat.

### Results

**Preclinical Studies.** CYP3A induction by semagacestat. After 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 four of the hepatic cytochrome preparations (HH969, HH970, HH975, and H0088) examined (Fig. 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 reported previously (Kostrub-
The increase in CYP3A-mediated TH activity was statistically significant for all hepatocyte preparations treated with 1 μM semagacestat and higher \((p < 0.05)\). 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 compared with activity in vehicle control incubations \((p < 0.05)\). Each preparation also showed a significant induction of CYP3A-mediated TH activity with the positive control rifampicin \((10 \mu M)\), 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 \(E_{\text{max}}\) for semagacestat was estimated to be 11.6-fold \((S.E. \text{ of the estimate, } 0.5)\), whereas \(EC_{50}\) was estimated to be 0.81 μM \((S.E. \text{ of the estimate, } 0.16)\). These values along with the average unbound steady-state concentration of semagacestat after daily dosing of 140 mg of approximately 0.37 μM \((\text{fraction unbound in plasma is } 0.7)\) predict a 4.6-fold increase in hepatic CYP3A content \((\text{Fahmi et al., } 2009)\).

**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 because...
of 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 of 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; Supplemental Fig. 1, a and b). During coadministration with semagacestat, the oral clearance of midazolam increased approximately 4.25-fold, whereas 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 after both a single 140-mg dose and multiple 140-mg doses for 10 days when coadministered with 400 mg of ketoconazole administered for 15 days (Supplemental Fig. 2, a and b). Compared with semagacestat alone, oral clearance of semagacestat was reduced 32% after a single dose and 46% after multiple doses when coadministered with ketoconazole (Table 4). After multiple doses of semagacestat, a higher semagacestat plasma clearance (31.3 l/h) was observed compared with plasma concentrations during midazolam- or nifedipine-only administration (Table 3; Supplemental Fig. 1, a and b). During coadministration with semagacestat, the oral clearance of midazolam increased approximately 4.25-fold, whereas the oral clearance of nifedipine increased approximately 3.65-fold (Table 2).

The AUC of semagacestat’s M3 metabolite (formed by CYP3A) increased approximately 4.25-fold, whereas the AUCs of both semagacestat and its M2 metabolite (not formed by CYP3A) increased during coadministration with ketoconazole (Table 4; Supplemental Fig. 3b). On the contrary, the AUC of the M2 metabolite was essentially unchanged after autoinduction by semagacestat (Table 4; Supplemental Fig. 3b).

After multiple doses of semagacestat, the AUC from time zero to 8 h of ketoconazole was reduced by approximately 50% (Table 5; Supplemental Fig. 4).

Safety (studies 1 and 2). One serious adverse event was observed, during which a subject experienced an anaphylactic reaction 5 min after first coadministration of semagacestat and ketoconazole; this subject was discontinued from study 2. A total of three 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 sequelae. 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 eight subjects developed rash (semagacestat plus midazolam, n = 3; semagacestat plus nifedipine, n = 2; semagacestat, n = 2; semagacestat plus ketoconazole, n = 1) and four subjects experienced pruritus (semagacestat plus midazolam, n = 1; semagacestat plus nifedipine, n = 1; semagacestat, n = 1; semagacestat plus ketoconazole, n = 1).
of the absorbed dose is oxidized by CYP3A4 and CYP3A5. Semagacestat was not predicted to be an inhibitor of any P450 enzyme or any transporter, according to 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 (Fig. 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

### 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>AUC0-tlast, ng · 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>AUC0-tlast, ng · h/ml</td>
<td>MDZ + SMG</td>
<td>20</td>
<td>23.2 (20.3, 26.5)</td>
<td>0.23 (0.19, 0.27)</td>
</tr>
<tr>
<td>Cmax, 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>Median tmax, h (range)</td>
<td>MDZ only</td>
<td>20</td>
<td>0.5 (0.3, 2.0)</td>
<td>N.A.</td>
</tr>
<tr>
<td>CL/F, l/h (range)</td>
<td>MDZ + SMG</td>
<td>20</td>
<td>76.3 (28.9)</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

### 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>AUC0-tlast, ng · h/ml</td>
<td>SMG only</td>
<td>20</td>
<td>6320 (5740, 6970)</td>
<td>1.46 (1.38, 1.56)</td>
</tr>
<tr>
<td>AUC0-tlast, ng · h/ml</td>
<td>SMG + KTZ</td>
<td>18</td>
<td>9260 (8400, 10200)</td>
<td>1.25 (1.06, 1.46)</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>SMG only</td>
<td>20</td>
<td>1640 (1420, 1900)</td>
<td>1.25 (1.06, 1.46)</td>
</tr>
<tr>
<td>CL/F, l/h</td>
<td>SMG</td>
<td>20</td>
<td>22.1 (20.1, 24.4)</td>
<td>0.68 (0.64, 0.73)</td>
</tr>
<tr>
<td>Multiple-dose SMG—effect on SMG</td>
<td>SMG only</td>
<td>20</td>
<td>4480 (4140, 4850)</td>
<td>1.84 (1.72, 1.97)</td>
</tr>
<tr>
<td>AUC0-tlast, ng · h/ml</td>
<td>SMG + KTZ</td>
<td>18</td>
<td>8230 (7580, 8930)</td>
<td>1.16 (1.06, 1.26)</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>SMG only</td>
<td>20</td>
<td>1280 (1130, 1440)</td>
<td>1.46 (1.26, 1.70)</td>
</tr>
<tr>
<td>CL/F, l/h</td>
<td>SMG</td>
<td>20</td>
<td>31.3 (28.9, 33.9)</td>
<td>0.54 (0.51, 0.58)</td>
</tr>
<tr>
<td>Effect on metabolite M2</td>
<td>SMG only</td>
<td>20</td>
<td>1800 (1650, 1960)</td>
<td>1.29 (1.20, 1.37)</td>
</tr>
<tr>
<td>Single-dose SMG AUC0-tlast, ng · h/ml</td>
<td>SMG</td>
<td>18</td>
<td>2320 (2120, 2530)</td>
<td>1.32 (1.22, 1.43)</td>
</tr>
<tr>
<td>Multiple dose SMG AUC0-tlast, ng · h/ml</td>
<td>SMG</td>
<td>18</td>
<td>1890 (1740, 2050)</td>
<td>1.32 (1.22, 1.43)</td>
</tr>
</tbody>
</table>

### Footnotes

1. AUC0-tlast area under the concentration versus time curve from time zero extrapolated to infinity; AUC0-tlast area under the concentration versus time curve during one dosing interval; AUCtlast, area under the concentration versus time curve during one dosing interval at steady state; CL/F, apparent total clearance at steady state; CL/F, apparent clearance at steady state; GMRE, geometric means ratio estimate; KTZ, ketoconazole; LS GeoMean, least-squares geometric mean; N, number of subjects; SMG, semagacestat.
2. Terminal slope could not be accurately determined in two subjects.
3. Terminal slope could not be accurately determined in four subjects.
4. Terminal slope could not be accurately determined in two subjects.
5. Terminal slope could not be accurately determined in two subjects.

### Additional References

- [Ayan-Oshodi et al.](http://www.aspetjournals.org)
- [KTZ once daily (400 mg), day 1 of period 1.](http://www.aspetjournals.org)
- [SMG single dose alone (140 mg), day 5 of period 2.](http://www.aspetjournals.org)
- [SMG single dose alone (140 mg), day 10 of period 1.](http://www.aspetjournals.org)
- [SMG once daily (140 mg) + KTZ once daily (400 mg), day 14 of period 2.](http://www.aspetjournals.org)
concentrations expected at steady state after 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 Cmax 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 Cmax 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 EC50 and dose of semagacestat is less than that associated with rifampicin liver.

given that the systemic clearance reflects the CYP3A activity in the reflects an increase in systemic clearance and would be expected to the same non-CYP3A value in both scenarios.

Ketoconazole exposure was markedly decreased after 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). Although 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 P450 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. There was a small (approximately 13%), although statistically significant, increase in semagacestat clearance after multiple doses were coadministered with ketoconazole. Although 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 P450 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.

**TABLE 5**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SMG SD + KTZ q.d.</th>
<th>SMG q.d. + KTZ q.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>4980 (259)</td>
<td>3330 (458)</td>
</tr>
<tr>
<td>tmax, h</td>
<td>2.00 (0.92, 4.00)</td>
<td>2.00 (0.92, 4.00)</td>
</tr>
<tr>
<td>AUC0−τ, ng·h/ml</td>
<td>25,100 (304)</td>
<td>12,700 (345)</td>
</tr>
</tbody>
</table>

AUC0−τ, area under the concentration versus time curve from time zero to 8 h; KTZ, ketoconazole (400 mg); N, number of subjects; SMG, semagacestat (140 mg); SD, single dose; tmax, time of Cmax.

*b Day 5 of period 2.

*b Day 14 of period 2.

*b Median (range).
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 whether autoinduction of semagacestat contributed to these events. The fourth discontinuation occurred as a 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, taken together, the data from the preclinical and clinical pharmacology studies 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. In addition, 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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Authorship Contributions
Participated in research design: Ayan-Oshodi, Willis, Annes, Lowe, Friedrich, de la Peña, Zhang, Brown, Wise, and Hall.

Conducted experiments: de la Peña and Wise.

Performed data analysis: Ayan-Oshodi, Willis, Annes, Lowe, Friedrich, Zhang, Brown, and Hall.

Wrote or contributed to the writing of the manuscript: Ayan-Oshodi, Willis, Annes, Lowe, Friedrich, de la Peña, Zhang, Brown, Wise, and Hall.

References


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.
Presented are means ± standard deviations.

Abbreviations: SMG=semagacestat (140 mg).
Nifedipine (60 mg).

Supplemental Figure 1b.
Supplemental Figure 2a.

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

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

![Graph showing plasma concentration of semagacestat over time with annotations for SMG QD (Period 1, Day 10) and SMG QD + KTZ QD (Period 2, Day 14).]

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

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

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.

The graph shows the plasma concentration of Ketoconazole over time for two conditions: SMG QD + KTZ QD (Period 2, Day 5) and SMG QD + KTZ QD (Period 2, Day 14). The x-axis represents time in hours (0, 2, 4, 6, 8), and the y-axis represents the plasma concentration in ng/mL (0 to 12,000). The red line with circles represents the first condition, while the blue line with triangles represents the second condition. The data points are accompanied by error bars indicating variability.