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ORIGINAL ARTICLE

Double-blind crossover study to assess potential differences in cytochrome P450 3A4 activity in healthy subjects receiving ondansetron plus dexamethasone, with and without aprepitant

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Abstract
Purpose Because glucocorticoids and the neurokinin-1 receptor antagonist aprepitant influence CYP3A4 activity, this study assessed whether aprepitant added to a 5-HT_3 antagonist and glucocorticoid would affect CYP3A4 induction.
Methods In this double-blind, 2-period crossover study, 12 subjects were randomized to receive a triple regimen (oral aprepitant [A] 125 mg, intravenous ondansetron [O] 32 mg, and oral dexamethasone [D] 12 mg day 1; A 80 mg and D 8 mg days 2–3; D 8 mg day 4) in 1 of 2 periods, and a dual regimen (O 32 mg and D 20 mg day 1; D 8 mg bid days 2–4); the D dose was adjusted to account for known dexamethasone/aprepitant interaction. Oral (2 mg) and intravenous (1 mg) stable isotope ($^{13}C_5$ $^{15}N_1$)–labeled midazolam were simultaneously given as probes on days −1, 6, 8, 15, and 22 of each period. If the a priori 90% confidence interval for the day 6 geometric mean oral midazolam $AUC_{0-\infty}$ ratio (triple/dual regimen) of fold-change from baseline was above 0.5, it would be concluded that there was no clinically meaningful between-regimen difference in CYP3A4 activity.
Results Day 6 oral midazolam $AUC_{0-\infty}$ geometric mean fold-change from baseline was 0.84 (0.30–1.58 with A, 0.46–1.69 without A). The ratio of geometric mean oral midazolam $AUC_{0-\infty}$ fold-changes was 1.00 (90% confidence interval 0.80, 1.25).
Conclusions Aprepitant plus a 5-HT_3 antagonist and dexamethasone is unlikely to have a significant additional inductive effect on CYP3A4 activity beyond that of the dual regimen.

Keywords Aprepitant · Midazolam · Ondansetron · CYP3A4 · Pharmacokinetics · Nausea/vomiting

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Introduction

Aprepitant is the first neurokinin-1 (NK_1) receptor antagonist recommended by several practice guidelines for use in combination with other antiemetics for the prevention of chemotherapy-induced nausea and vomiting (CINV) [1–3] and as monotherapy for the prevention of postoperative nausea and vomiting [4]. Because many drugs, including some used in chemotherapy regimens, are metabolized in whole or in part by the CYP3A4 system, the potential effects of aprepitant on this enzyme system were evaluated in a previous study [5]. In that study, the recommended 3-day regimen of aprepitant (aprepitant 125 mg on day 1 and aprepitant 80 mg on days 2 and 3) or placebo was given with oral midazolam. Aprepitant had a weak inhibitory effect (25% increase in midazolam area under the plasma concentration–time curve [$AUC_{0-\infty}$]) on day 4, a weak inductive effect (19% decrease in midazolam $AUC_{0-\infty}$) on day 8, and no significant effect on day 15. In another study that used midazolam probing to investigate the effects on

CYP3A4 of aprepitant 80 mg given once daily for 36 days, aprepitant induced CYP3A4 activity, with a decrease in the midazolam ratio of geometric mean fold-change (aprepitant/placebo) to 39% on day 41 (data on file). No inductive effect was observed on day 75 (the recovery phase of the study). Additionally, aprepitant administered as a single oral dose of 125 mg in a third study had no clinically meaningful effect on the pharmacokinetics of coadministered intravenous (IV) midazolam [6].

In addition to 3 days of dosing with aprepitant, the triple therapy regimen recommended for antiemetic prophylaxis in patients receiving chemotherapy includes a 5-HT_3 antagonist, such as ondansetron, and a glucocorticoid, such as dexamethasone. Although prior data have shown that aprepitant does not interact significantly with at least four different 5-HT_3 antagonists [7–9], the potential inductive effects of dexamethasone on CYP3A4 activity in humans have not been thoroughly evaluated [10]. Supportive-care doses of dexamethasone (16–24 mg/day for 2–9 days, or 8 mg orally twice daily for 5 days) have been shown to increase hepatic CYP3A4 catalytic activity by 55 and 26%, respectively [11, 12]. These increases were estimated by changes in the erythromycin breath test (EBT), a generally accepted probe for CYP3A4 activity [13]. Among the limited data available with midazolam, a sensitive probe with broader utility than the EBT in quantifying CYP3A4 activity [14, 15], IV midazolam given to patients on chronic glucocorticoids resulted in a 64% decrease in midazolam $AUC_{0-\infty}$ with a 128% increase in midazolam clearance [16]. The potential impact of dexamethasone on oral midazolam is not well understood; one study with midazolam as a probe showed that dexamethasone 2 or 5 mg once daily for 7 days did not significantly induce CYP3A4 activity [17], but there is a scarcity of additional corroborating research.

Thus, given (1) the inconclusive evidence regarding potential effects of dexamethasone on CYP3A4, (2) the known CYP3A4-inductive effect of aprepitant given as monotherapy [5], (3) the known inhibitory effect of aprepitant on CYP3A4-mediated metabolism of dexamethasone, and (4) the inclusion of both aprepitant and dexamethasone in the triple therapy regimen used clinically for CINV, the potential effects on CYP3A4 of aprepitant in combination with dexamethasone warranted further characterization. In order to assess whether aprepitant had a clinically meaningful inductive effect over and above that of dexamethasone given without aprepitant, the study was designed to compare the effects on CYP3A4 activity of dual therapy (ondansetron plus dexamethasone at the clinically recommended doses) versus triple therapy (ondansetron, dexamethasone, and aprepitant at the clinically recommended doses), i.e., dual therapy with and without coadministered aprepitant. The dexamethasone dose was adjusted to achieve similar dexamethasone exposure when given with and without aprepitant [4]. In addition, oral and IV midazolam were administered simultaneously to allow an independent assessment of both intestinal and hepatic CYP3A4 activity.

Methods

Design and procedures

In this double-blind, 2-period study, subjects were randomized to 1 of 2 groups according to a randomization schedule generated by the sponsor. According to the crossover design of the study, Group I received Treatment A in the first period and Treatment B in the second period; Group II received Treatment B followed by Treatment A (Table 1). Treatment A (triple regimen) consisted of aprepitant 125 mg administered orally (PO), ondansetron 32 mg IV, and dexamethasone 12 mg PO on day 1; aprepitant 80 mg and dexamethasone 8 mg PO on days 2 and 3; and dexamethasone 8 mg on day 4. Treatment B (dual regimen) consisted of ondansetron 32 mg IV and dexamethasone 20 mg PO on day 1, and dexamethasone 8 mg twice daily on days 2, 3, and 4. Matching placebos for aprepitant and for dexamethasone were used to maintain blinding. Due to the known inhibitory effect of aprepitant on CYP3A4-mediated metabolism of dexamethasone, the recommended clinical dose of dexamethasone in the triple antiemetic regimen, as described in the product label, is lower than in the dual regimen in order to achieve dexamethasone exposure comparable to that of the dual antiemetic regimen [4] As aprepitant does not affect ondansetron pharmacokinetics, the same dose of ondansetron was used in both treatments [7].

To compare the effects of the treatments on systemic versus first-pass CYP3A4 activity, single doses of oral (2 mg) unlabeled midazolam and IV (1 mg) stable isotope ($^{13}C_5$ $^{15}N_1$)-labeled midazolam were simultaneously administered as probes on days −1 (baseline), 6, 8, 15, and 22 of each period. A washout period of at least 14 days was required between the last day of midazolam probing in period 1 (day 22), and the first day of midazolam probing in the second period (day −1).

Blood samples for plasma midazolam assay were obtained in Periods 1 and 2 on days −1 (baseline), 6, 8, 15, and 22 at the following time points, relative to IV midazolam administration: predose (days −1 and 8 only); 2 min (end of IV push of midazolam); 15 min; 30 min; 1, 2, 3, 4, 6, 8, 12, and 24 h following IV midazolam administration.

Rationale for endpoints and hypotheses

The primary measure of interest was the oral midazolam $AUC_{0-\infty}$ fold-change from baseline (day −1). The secondary measure of interest was the IV midazolam $AUC_{0-\infty}$

Table 1 Treatment regimens

Treatment[a]	Hour	Day 1	Days 2 and 3	Day 4
A	0	Aprepitant 125 mg, PO	Aprepitant 80 mg, PO	–
	0.5	Ondansetron 32 mg, IV	–	–
	0.5	Dexamethasone 12 mg, PO	Dexamethasone 8 mg, PO	Dexamethasone 8 mg, PO
	0.5	Placebo (dexamethasone) PO	–	–
	12.5	–	Placebo (dexamethasone) PO	Placebo (dexamethasone) PO
B	0	Placebo (aprepitant) PO	Placebo (aprepitant) PO	–
	0.5	Ondansetron 32 mg, IV	–	–
	0.5	Dexamethasone 20 mg, PO	Dexamethasone, 8 mg PO	Dexamethasone, 8 mg PO
	12.5	–	Dexamethasone, 8 mg PO	Dexamethasone, 8 mg PO

IV intravenous, *PO* per os (oral)

[a] For both treatment groups, oral (2 mg) and intravenous (1 mg) midazolam were administered simultaneously at $t = 0$ on days −1, 6, 8, 15, and 22

fold-change from baseline (day −1). The primary comparison of interest was the triple antiemetic regimen versus the dual antiemetic regimen on day 6.

The $AUC_{0-\infty}$ fold-change from baseline was calculated as [day AUC/baseline AUC], for days 6, 8, 15, and 22. Day 6 was selected as the primary time point of interest, as it was expected to represent the point of maximum CYP3A4 induction. This expectation was based on previous findings, which showed that during aprepitant administration, a CYP3A4-inhibitory effect initially predominates, followed by clearance of aprepitant from the plasma, at which time CYP3A4-inductive effects then predominate [5]. In addition, it was anticipated that the highest midazolam exposure was likely to be at baseline, with any treatment likely to decrease exposure at the time points to be examined. A 50% or greater reduction in oral midazolam $AUC_{0-\infty}$ ratio (triple antiemetic regimen/dual antiemetic regimen) of fold-change from baseline was considered to be clinically meaningful in this study, based on the results observed with St. John's wort, which has been reported to reduce midazolam AUC ~55% after oral midazolam, and ~20% after IV midazolam [18]. Thus, the primary hypothesis was that the true geometric mean ratio (GMR) (with aprepitant/without aprepitant) of true mean fold-changes in oral midazolam $AUC_{0-\infty}$ on day 6 would be ≥0.5 to indicate not clinically relevant. The secondary hypothesis was that the true GMR (with aprepitant/without aprepitant) of true mean fold-changes in stable isotope–labeled IV midazolam $AUC_{0-\infty}$ on day 6 would likewise be clinically not meaningful; i.e. the ratio would be ≥0.5.

Midazolam fold-changes from baseline on days 8, 15, and 22 were also assessed. Day 8 corresponded with the initiation of subsequent cycles of weekly chemotherapy relative to day 1 of the first cycle, and day 15 represented the time point at which the CYP3A4-inductive effects of aprepitant (without ondansetron and dexamethasone) were observed to have returned to baseline [5]. Day 22 was evaluated to confirm resolution of any potential inductive effects of the triple antiemetic regimen on oral midazolam.

Tolerability of the treatments was assessed with complete prestudy and post-study physical examinations, including vital signs, routine hematology and chemistry tests, urinalysis, and 12-lead electrocardiograms, as well as continuous monitoring for adverse events throughout the study.

Variance estimates from two previous sponsor studies were used to power the study [5, 6]. The true within-subject variance (natural log-scale) of oral midazolam $AUC_{0-\infty}$ was assumed to be 0.1814; the true within-subject variance of IV midazolam $AUC_{0-\infty}$ was assumed to be 0.0508. On day 6, given a 2-period crossover design with repeated measures, a one-sided test, $\alpha = 0.05$, and 12 subjects completing the study in each treatment period, there was a 0.98 probability that the 90% confidence interval (CI) for the true oral midazolam $AUC_{0-\infty}$ GMR of fold-changes from baseline (day 6/day −1) would be >0.5, given a true ratio of 1. The probability that the 90% CI for the true IV midazolam $AUC_{0-\infty}$ GMR of fold-changes from baseline (day 6/day −1) would be >0.5 was at least 0.99, given a true ratio of 1.

Subjects

This single-center trial was performed at the Thomas Jefferson University (TJU) Clinical Research Unit (Philadelphia, PA) in conformance with legal requirements for the ethical conduct of research in human subjects, and approval was obtained from the TJU Institutional Review Board. All subjects gave written informed consent to participate. Eligible subjects were healthy, nonsmoking adult males and females between the ages of 18 and 45 years who were within 30% of ideal body weight for age, gender, and height. Females of childbearing potential were required to have a negative prestudy serum β-HCG test and were required to use

double-barrier contraception for 28 days after study start. Subjects discontinued use of any prescription or nonprescription drugs at least 3 weeks prior to the baseline administration of midazolam (i.e., day −1) and for the duration of the study. This restriction applied especially to drugs or foods known to influence CYP3A4, including but not limited to clarithromycin, erythromycin, ketoconazole, nefazodone, indinavir, saquinavir, cyclosporin, St. John's wort, grapefruit and grapefruit juice, garlic extract, rifampin, glucocorticoids, and anticonvulsants.

Midazolam and aprepitant supplies

Stable isotope–labeled midazolam was prepared in seven steps, utilizing the following commercially available labeled intermediates: Ethyl [$^{13}C_2$,^{15}N]glycine, [^{13}C]nitromethane, and [$^{13}C_2$]acetaldehyde (Cambridge Isotope Labs, Andover MA and/or Isotec Inc., Miamisburg OH).

Commercially available benzophenone was condensed with ethyl [$^{13}C_2$,^{15}N]glycine using the procedure of Van Dort et al. [19]. Incorporation of [^{13}C]nitromethane was then carried out to afford the intermediate in three steps [20]. Conversion of the intermediate to midazolam was conducted via the procedure of Walser et al. [21] through the intermediacy of a nitroxime and condensation with [$^{13}C_2$] acetaldehyde. Approximately 500 mg of [$^{13}C_5$,^{15}N]midazolam (>99% M + 6) was prepared in a singe batch via this procedure. The identity was verified by high-performance liquid chromatography (HPLC), liquid chromatography/mass spectrometry (LC/MS), and nuclear magnetic resonance (NMR) comparison with reference unlabeled midazolam. Aprepitant was provided by Merck and Co., Inc., the sponsor of the study.

Plasma midazolam assays

Samples collected for stable isotope–labeled and unlabeled midazolam assays were analyzed using liquid–liquid extraction for analyte isolation followed by LC-tandem mass spectrometry (MS/MS) detection. Plasma midazolam assay validation and sample analysis were conducted in accordance with applicable standard operating procedures of the laboratory that performed the analyses (Taylor Technology, Inc., Princeton, NJ). Duplicate quality control samples were run daily at three concentrations: low (30 ng/ml), mid (2,500 ng/ml), and high (4,500 ng/ml). The analysis was completed in 2 days. The mean calculated concentrations of the low-, mid-, and high-quality control samples were 30.8, 2,563.2, and 4,732.9 ng/ml, respectively. The accuracy and precision (coefficient of variation) of the quality control samples were 103% (3.2%), 103% (2.1%), and 102% (5.7%) for low-, mid-, and high-quality control samples, respectively.

Midazolam and [$^{13}C_5$,^{15}N]midazolam [(m + 6) midazolam] were extracted from 0.5 ml of heparinized human plasma containing 10 ng of an internal standard, [$^{13}C_3$] midazolam, in 1.0 ml of 0.1 M sodium borate buffer and 3.0 ml of methyl t-butyl ether was added to each tube, following the addition of 50 µl of 0.01 N hydrochloric acid to each sample. Samples were placed on a reciprocating shaker for 15 min and then centrifuged at 3,000 rpm for another 15 min. Sample tubes were placed in a dry ice/acetone bath until the aqueous portion was frozen; then, the organic layer was decanted into a clean 12 × 75 mm glass culture tube. Samples were evaporated to dryness under nitrogen at 40–50°C for approximately 30 min, then reconstituted in 60 µl of methanol and sonicated for approximately 1 min and vortexed. The methanolic extracts were then transferred to polypropylene autosampler vials containing 90 µl of 1.5 mM ammonium acetate in 0.05/100, v/v acetic acid/water.

The extracts (20–60 µl) were chromatographed on a Hewlett Packard Series II 1090L LC, under reverse-phase conditions, using a Genesis C18 3-mm, 3-μ column, fitted with a Keystone Beta Basic C18 guard column. The column oven temperature was set to 50°C ± 5°C. The initial flow rate was 0.6 ml/min. The mobile phase solutions were 1.5 mM acetate in 0.5/1,000, v/v acetic acid/water (mobile phase A) and pure HPLC grade methanol (mobile phase B). Elution of unlabeled midazolam, [$^{13}C_5$,^{15}N]midazolam, and internal standard was achieved using the following gradient (%B): 50% from 0 to 2 min, 70% from 2 to 3.4 min, 75% from 3.4 to 3.5 min, 100% from 3.4 to 3.5 min, and 50% from 3.5 to 4.8 min. The total run time was 5.0 min.

The compounds were detected and quantified by HPLC–atmospheric pressure chemical ionization (APCI)/MS/MS in positive ion mode using a Finnigan TSQ-7000 or TSQ-700 (Finnigan Corp., San Jose, CA) equipped with their standard APCI source. The heated capillary setting was 275°C; the corona was 5 μamps, and the collision energy was −28 eV. The mass spectrometer was operated in the positive ion daughter mode with the manifold pressure between 1.8 and 2.5×10^{-5} torr with the collision gas turned on. MS/MS monitoring of the precursor/product ion transitions was carried out. The precursor → product ion combinations of *m/z* 332.1 → 297, 329.1 → 294, and 326.1 → 291 were used to quantify [$^{13}C_5$,^{15}N]midazolam, [$^{13}C_3$]midazolam, and midazolam, respectively. A linear function was fit to the calibration data using a 1/(concentration)^2 weighted least squares regression procedure. The lower limit of quantitation was 0.100 ng/ml.

Individual plasma unlabeled and stable isotope–labeled midazolam concentrations were used to estimate the area under the plasma midazolam concentration–time curve from time zero to infinity ($AUC_{0-\infty}$). Estimates were made for prestudy (day −1) and on days 6, 8, 15, and 22 using

noncompartmental analysis with the commercial software WinNonlin Enterprise Version 4.1 (Pharsight Corporation, Mountain View, CA). A weight of $1/y$ was used for fitting the terminal natural log-linear portion of the plasma concentration–time curve.

Plasma aprepitant assays

Blood samples for measurement of aprepitant plasma concentration were obtained prior to midazolam dosing on day −1 (Period 1 only) and 24 h following aprepitant dosing on days 1, 2, and 3 (Periods 1 and 2) (data not shown). Exact times of all sample collections were recorded. Samples collected for aprepitant assay were analyzed by the sponsor. The analytical method used liquid–liquid extraction for analyte isolation followed by LC–MS/MS detection.

Statistical methods

The sponsor managed the data and performed the statistical analyses. Per protocol, the primary hypothesis was addressed by evaluating individual natural log-transformed oral midazolam $AUC_{0-\infty}$ values with a linear mixed effects model having fixed effects for sequence, period, day, treatment, and treatment-by-day interaction, and a random effect for subject within sequence. A two-sided 90% CI for the true $AUC_{0-\infty}$ GMR (with aprepitant/without aprepitant) of fold-change from baseline on day 6 was calculated from the model, using the within-subject residual error and referencing a t-distribution. If the lower limit of the 90% CI was ≥ 0.5 (i.e. a reduction no greater than 50%), then the hypothesis of no clinically meaningful between-treatment difference would be supported. The secondary hypothesis was addressed by analyzing the day sixfold-change in IV midazolam $AUC_{0-\infty}$ in a similar fashion. Two-sided P values for the between-treatment comparisons of fold-change from baseline were also calculated. Results for which $P \leq 0.050$ are reported as statistically significant. P values between 0.05 and 0.10 are referred to as having approached significance. No multiplicity adjustments were required.

Results

The study enrolled 12 subjects with a mean age of 34 years (range 22–44 years). Ten of the subjects were Caucasian (83%) and ten of the subjects were male (83%). All 12 subjects completed the study, and there were no statistically significant sequence or period effects.

Midazolam

The arithmetic mean oral midazolam plasma concentration profiles following Treatment A (triple regimen with aprepitant) or Treatment B (dual regimen without aprepitant) are shown in Fig. 1. Table 2 shows plasma midazolam AUC results following Treatments A and B with oral and IV midazolam probes. Figure 2 shows individual and geometric

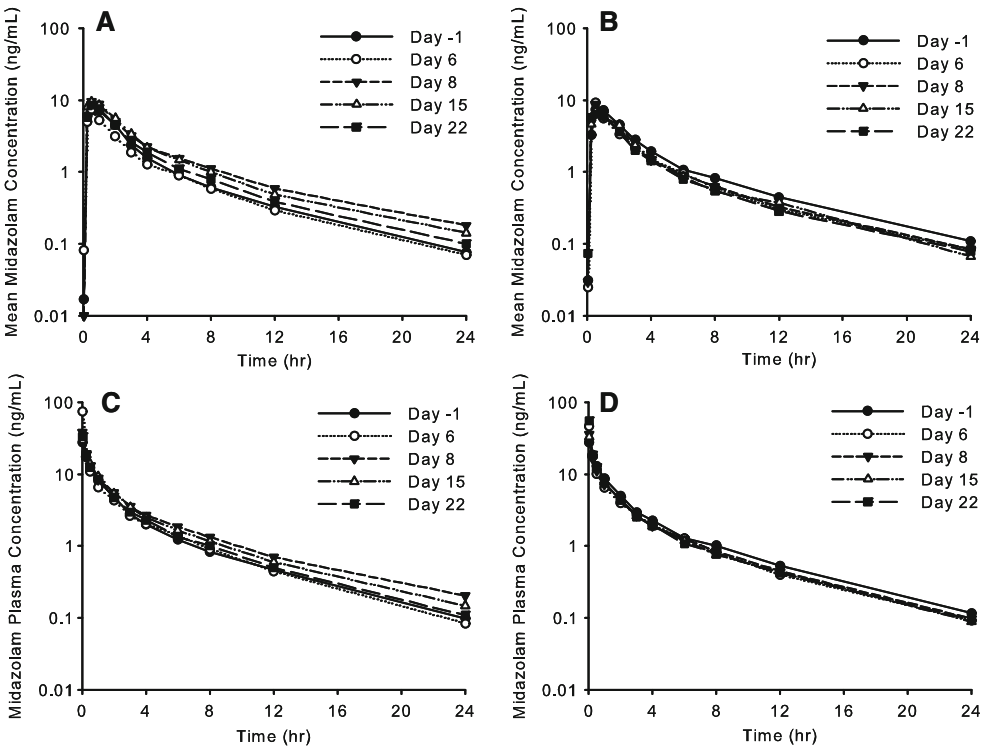

Fig. 1 Mean plasma concentration profiles of midazolam following 2-mg oral dose in healthy subjects ($N = 12$) who received the dual therapy regimen (**a**) or triple therapy regimen (**b**). Mean plasma concentration profiles of midazolam following 1-mg IV dose in healthy subjects ($N = 12$) who received the dual therapy regimen (**c**) or triple therapy regimen (**d**)

Table 2 Plasma midazolam AUC results following Treatment A (aprepitant/ondansetron/dexamethasone) and Treatment B (ondansetron/dexamethasone), with oral and intravenous midazolam probes ($N = 12$)

Probe	Day	Average midazolam $AUC_{0-\infty}$ (SD)		Midazolam $AUC_{0-\infty}$ GM fold-change from baseline (day/baseline) (SD)		Ratio of GM fold-changes, with/without aprepitant (90% CI)	*P* value
		Treatment A	Treatment B	Treatment A	Treatment B		
Oral midazolam							
$AUC_{0-\infty}$ (ng h/ml)	Baseline	20.9 (11.2)	19.8 (6.3)	–	–	–	
	6	17.5 (9.6)	16.6 (6.8)	0.84 (0.39)	0.84 (0.35)	1.00 (0.80, 1.25)	>0.250
	8	19.0 (7.2)	28.8 (12.9)	0.91 (0.40)	1.45 (0.52)	0.63 (0.49, 0.78)	<0.010
	15	19.4 (5.8)	27.8 (12.4)	0.93 (0.36)	1.40 (0.54)	0.66 (0.53, 0.83)	<0.010
	22	17.3 (5.7)	23.4 (7.6)	0.83 (0.44)	1.18 (0.35)	0.70 (0.56, 0.88)	<0.010
IV midazolam							
$AUC_{0-\infty}$ (ng h/ml)	Baseline	39.5 (12.3)	38.1 (7.6)	–	–	–	
	6	35.5 (9.7)	39.0 (9.4)	0.90 (0.23)	1.02 (0.18)	0.88 (0.74, 1.03)	0.184
	8	36.8 (8.4)	47.7 (14.1)	0.93 (0.20)	1.25 (0.35)	0.74 (0.63, 0.87)	<0.010
	15	35.6 (7.2)	43.6 (13.7)	0.90 (0.21)	1.14 (0.36)	0.79 (0.67, 0.93)	0.017
	22	37.2 (9.3)	40.8 (5.0)	0.94 (0.34)	1.07 (0.30)	0.88 (0.75, 1.04)	0.198

$AUC_{0-\infty}$ area under the plasma concentration–time curve, *GM* geometric mean, *SD* standard deviation, *P-value* two-sided *P* value for between-treatment comparison of fold-change from baseline

mean ratios (GMRs) (triple regimen/dual regimen) of oral and IV midazolam $AUC_{0-\infty}$ fold-change from baseline. On day 6, the oral midazolam $AUC_{0-\infty}$ geometric mean fold-changes from baseline when subjects received the triple regimen, and when the same subjects received the dual regimen, were both 0.84. The ratio (with aprepitant/without aprepitant) of geometric mean oral midazolam $AUC_{0-\infty}$ fold-changes (90% CI) was 1.00 (0.80, 1.25). Similarly, the IV midazolam $AUC_{0-\infty}$ geometric mean fold-changes from baseline on day 6 were 0.90 when subjects received the triple regimen, and 1.02 when the same subjects received the dual regimen (Table 2). The ratio (with aprepitant/without aprepitant) of geometric mean IV midazolam $AUC_{0-\infty}$ fold-changes (90% CI) was 0.88 (0.74, 1.03). For both the primary and secondary measures, the CIs fell completely above the lower bound of 0.5 ($P < 0.05$), supporting the respective hypotheses (Table 2).

In addition, as shown in Table 2, the day-8 oral midazolam $AUC_{0-\infty}$ geometric mean fold-changes from baseline for the triple regimen and dual regimen were 0.91 and 1.45, respectively, and the GMR of oral midazolam $AUC_{0-\infty}$ fold-changes (90% CI) was 0.63 (0.49, 0.78) (Fig. 2). The corresponding oral midazolam $AUC_{0-\infty}$ geometric mean fold-changes from baseline on day 15 were 0.93 (triple regimen) and 1.40 (dual regimen), and the GMR of oral midazolam $AUC_{0-\infty}$ fold-changes (90% CI) was 0.66 (0.53, 0.83). On day 22, the oral midazolam $AUC_{0-\infty}$ geometric mean fold-changes from baseline were 0.83 (triple regimen) and 1.18 (dual regimen), and the GMR for oral midazolam $AUC_{0-\infty}$ fold-changes (90% CI) was 0.70 (0.56, 0.88). Table 2 shows results for IV midazolam.

Tolerability

There were no serious adverse events, discontinuations due to adverse events, or deaths. Nine of the 12 subjects reported a total of 24 nonserious clinical adverse events; 16 of these were judged by the investigator to be at least possibly related to the study drug. One nonserious laboratory adverse event occurred, but was considered probably not related to study drug.

Discussion

The NK1 antagonist aprepitant and the glucocorticoid dexamethasone are included in the triple therapy antiemetic regimen currently used for the prevention of CINV (aprepitant, dexamethasone, and a $5HT_3$ antagonist) [1–4]. Dexamethasone is a known inducer of CYP3A in rats [22] and human hepatocytes [10], but evaluations of its potential CYP3A4-inductive effects in vivo in humans are limited [10]. Aprepitant is known to have a weak inhibitory effect on CYP3A4 activity within 4 days of dosing, followed by a weak inductive effect at day 8 [5]. In addition, aprepitant has been shown specifically to inhibit the CYP3A4-mediated metabolism of dexamethasone [23]. Because studies have not thoroughly assessed the full extent and the duration of the inductive effect on CYP3A4 that may occur when aprepitant and dexamethasone are coadministered, this study was conducted to evaluate the potential impact on CYP3A4 activity resulting from the addition of aprepitant to ondansetron and dexamethasone.

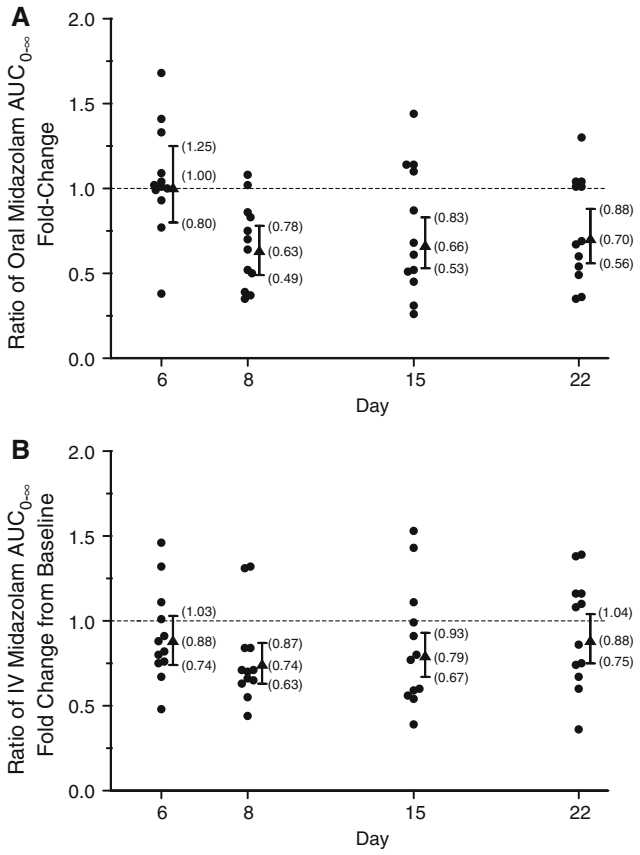

Fig. 2 Individual and geometric mean ratios (triple regimen/dual regimen) of oral (**a**) or intravenous (**b**) midazolam $AUC_{0-\infty}$ fold-change from baseline for 12 healthy subjects. *Filled circle* individual ratio, *filled triangle* geometric mean ratio (with 90% confidence interval indicated as *error bars*)

To test whether aprepitant either increases or decreases the toxicity of antineoplastic agents and rather than test multiple antineoplastic drugs, we used midazolam as a selective probe for CYP3A4 activity. The doses of dexamethasone used in this study differed for the two regimens in order to reflect the recommended dosing per label for the CINV indication, which accounts for the known interaction between aprepitant and dexamethasone. The ≥50% reduction in midazolam AUC ratio (triple therapy regimen/dual therapy regimen) was selected as indicative of clinical relevance based on the reduction in oral midazolam AUC on the order of 50% or greater seen with other agents such as St. John's wort, which has been reported to reduce midazolam AUC ~55% when midazolam is administered orally and ~20% when midazolam is given IV [24]. Modest reductions in midazolam AUC of ~20% following intravenous administration are likely to be of little clinical consequence, particularly with IV cancer chemotherapeutics where there is marked interindividual variability [25]. Modest reductions in midazolam AUC of ~26% have been reported with pioglitazone, though such changes are not necessarily considered clinically significant [25].

When the two regimens were compared with each other, a statistically significant decrease in the ratio of the fold-change from baseline in midazolam AUC was noted after day 8. This difference appeared to be due to an increase in midazolam AUC (45% after oral midazolam probing and 25% after intravenous midazolam probing on day 8) observed with the dual regimen. The effect persisted on days 15 and 22 and was consistent with weak CYP3A4 inhibition. This finding suggests that a slight inductive effect of aprepitant in the triple regimen may have counteracted the weak inhibitory effect of the dual regimen, ultimately resulting in no net clinically meaningful effect on CYP3A4 activity.

The weak inhibitory effect on CYP3A4 activity of the dual regimen in this study was unanticipated because glucocorticoids such as dexamethasone are reported to be inducers of CYP3A4 [12]. In patients taking chronic glucocorticoid therapy, the AUC of midazolam following IV dosing was 36% lower compared with patients not taking glucocorticoids, consistent with a modest CYP3A4 inductive effect of chronic glucocorticoid therapy [16]. However, other data do not corroborate this; a lack of significant effect of dexamethasone (either 2 or 5 mg/day for 7 days) on midazolam clearance following IV midazolam suggests that short-term, high-dose dexamethasone therapy does not significantly induce CYP3A4 in humans [26]. The mechanism for the weak CYP3A4 inhibitory effect of the dual regimen is not apparent since neither dexamethasone nor ondansetron is known to inhibit CYP3A4, and the inhibitory effect was evident only at time points beyond when either drug would have been completely cleared from the body.

While not statistically significant, intersubject variability in the change in CYP3A activity was noted in this study, as indicated in Fig. 2. Although this finding is not unexpected, it suggests that some patients (17–50%) may experience net increased gastrointestinal and/or hepatic CYP3A4 activity with the triple therapy relative to the dual regimen beginning on day 8.

A limitation of this study is that it did not compare the reductions in midazolam to the inductive effects of dexamethasone on midazolam, although such effects have not been thoroughly studied either alone or in combination with other agents. In addition, because CYP3A4 inhibition/induction was evaluated in healthy young adults rather than in cancer patients, this study did not address the effects of aprepitant on chemotherapeutic toxicities. Although cytokine therapy and other chemotherapeutic agents are known to affect CYP3A4 activity, our current results suggest that aprepitant is unlikely to contribute to cancer therapy-induced inhibition/induction of CYP3A4.

Simultaneous administration of oral unlabeled midazolam and intravenous stable isotope–labeled ($^{13}C5^{15}N1$) midazolam probes allowed assessment of first-pass (intestinal and hepatic) effect on CYP3A4 activity following oral administration and absorption, compared with direct administration into the systemic circulation. This approach has been previously employed to estimate hepatic and intestinal CYP3A4 activity without the temporal changes in enzyme activity and blood flow that have confounded previous studies [27]. Orally administered midazolam was used to gauge the impact of the combination of aprepitant and dexamethasone on orally administered CYP3A4 substrates, while intravenous midazolam was selected to estimate the impact on parenterally administered compounds metabolized by CYP3A4. The present study used three distinct isotopic forms of midazolam to distinguish oral and intravenous CYP3A4 substrate from the internal standard used in the bioanalytical assay. This technique provided a powerful experimental clinical protocol for characterizing a comprehensive profile of CYP3A4-mediated drug interactions. On day 6, at which time the weak inductive effect of aprepitant was expected to be at a maximum, there were no significant differences between the triple and dual antiemetic regimens with regard to effects on midazolam AUC following either oral or IV midazolam. The regimens both with and without aprepitant each resulted in a 16% decrease in midazolam AUC following oral administration. Intravenous midazolam probing showed a 10% decrease in midazolam AUC with the triple regimen and no effect with the dual regimen. Both decreases were well below the 50% or greater reduction in midazolam $AUC_{0-\infty}$ ratio of fold-change from baseline prespecified as likely to be clinically relevant. In summary, the results of this study support a lack of clinically relevant effect on CYP3A4 activity attributable to aprepitant, when it is coadministered with ondansetron and dexamethasone.

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