PHARMACOKINETICS AND BRAIN PENETRATION OF CASOPITANT, A POTENT AND SELECTIVE NK-1 RECEPTOR ANTAGONIST, IN THE FERRET

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Running title: Pharmacokinetics and brain penetration of casopitant in ferrets

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Non-standard abbreviations:

amu atomic mass unit
Ki binding affinity constant
Kd equilibrium dissociation constant
m/z mass to charge ratio
CL/F apparent total clearance
Vss/F apparent volume of distribution at steady-state
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ABSTRACT:
The pharmacokinetics and brain penetration of the novel NK-1 receptor antagonist casopitant (GW679769) were examined in ferrets. The ferret is known to respond to the full spectrum of agents recognized to induce emesis in humans, and the cisplatin-induced emesis models in the ferret have been used to establish the antiemetic potential of casopitant. Following single intraperitoneal dosing to the ferret, casopitant was rapidly absorbed, with plasma and brain concentrations being approximately equal at 2 hours post-dose. The predominant radioactive component present in the ferret brain after a single dose of [14C]casopitant was parent compound, accounting for approximately 76% of the radioactivity. The major metabolites present in brain tissue following administration of [14C]casopitant were hydroxylated casopitant (M1), and the corresponding ketone product of the M1 metabolite (M2), which accounted for approximately 19% and 3% of the radioactivity in the brain extracts, respectively. All three molecules had relatively similar potency against both ferret and human brain cortical NK-1, suggesting that the pharmacologic activity of casopitant in the ferret is largely attributable to parent compound and to a lesser extent, to its oxidative metabolites. Because casopitant is intended to be administered in combination with ondansetron, and because therapeutic synergy has been observed with this combination in the ferret, a drug interaction study was conducted. The additional pharmacodynamic benefit of the combination dose was not due to an alteration in the pharmacokinetics of either agent, but is likely the result of the complementary mechanisms of pharmacologic action of the two drugs.
The tachykinins are a family of peptide neurotransmitters which mediate the release of intracellular calcium by binding to a group of transmembrane receptors called neurokinins (NK). Neurokinin-1 (NK-1) receptor antagonists are believed to confer antiemetic activity by suppressing the activity of the nucleus tractus solitarius (NTS), which is the point at which the vagal afferents from the gastrointestinal tract converge with inputs from the area postrema and other regions of the brain believed to be important in the control and initiation of emesis. Casopitant (GW679769) is a potent and selective NK-1 receptor antagonist. It is currently in Phase III clinical development for the treatment of chemotherapy induced nausea and vomiting as well as post-operative nausea and vomiting. Previous animal and human studies have demonstrated that casopitant has antiemetic activity in vivo (King, 2006; Arpornwirat et al, 2006; Rolski et al, 2006).

The ferret is known to respond to the full spectrum of agents recognized to induce emesis in humans (King, 1990). The cisplatin-induced acute and delayed emesis models in the ferret have been used extensively to identify the antiemetic potential of novel drug therapies, including casopitant. In these models, casopitant has demonstrated a prolonged effect in the attenuation of cisplatin-induced emesis. This suggests that casopitant may be helpful in treating human patients undergoing chemotherapy who experience persistent nausea and vomiting for several days after drug treatment. Brain penetration and subsequent receptor occupancy are expected to be important factors which influence the onset and duration of the pharmacologic effects of casopitant. It is not known if the major metabolites of casopitant have NK-1 antagonist activity, or if the prolonged pharmacodynamic effects of casopitant observed in the ferret are due to
casopitant itself, one of its metabolites, or a combination. A better understanding of the pharmacokinetics and drug metabolism in the ferret is essential to elucidating the pharmacodynamic contribution of casopitant and its circulating metabolite(s) to the therapeutic effects observed in ferret emesis models.

Selective NK-1 receptor antagonists provide incremental improvement in efficacy when used in combination with 5-HT3 antagonists, both in preclinical animal models and in the clinical setting (Campos et al, 2001; Navari et al, 1999). In the model of chemotherapy-induced emesis in the ferret, ondansetron (a 5-HT3 antagonist) and casopitant have demonstrated therapeutically synergistic effects when administered concomitantly (Gagnon and King, 2006). It is not understood whether this pharmacodynamic synergy is the result of a pharmacokinetic drug-drug interaction, or the complementary mechanisms of action of the two drugs.

To better understand the disposition, and thereby the pharmacology of casopitant, studies were conducted to investigate the pharmacokinetics, metabolism, and brain penetration of casopitant in the male ferret following single dose administrations. Because pharmacodynamic synergy has previously been observed following concomitant administration of casopitant with ondansetron, a three-way crossover drug interaction study was also performed in ferrets to determine whether concomitant administration of casopitant with ondansetron alters the pharmacokinetics of either agent. The in vivo metabolism of ondansetron in humans is predominantly mediated by CYP3A4 (GlaxoSmithKline, 2006), while the elimination of casopitant is largely mediated by CYP3A4 in humans (data on file). No information is available regarding the biotransformation of ondansetron or casopitant in the ferret.
METHODS:

Chemicals, Reagents and Equipment

Casopitant, [14C]casopitant, [3H]-GR205171, GR205171A, GSK329530A, GSK1497960A and ondansetron were synthesized by GlaxoSmithKline. CycloneP TurboFlow columns (0.5x50 mm; Thermo Scientific, Franklin, MA) and Synergi Polar-RP columns (2x50 and 2x100 mm; 4µ; Phenomenex, Torrance, CA) were used in the bioanalysis of samples by LC/MS/MS. A Zorbax RX-C18 column (4.6 x 150 mm, Agilent Life Sciences, Santa Clara, CA) was used to separate analytes for the metabolite identification work. Ammonium formate, formic acid, acetonitrile, Ultima Gold XR scintillation cocktail, and ammonium acetate were obtained commercially and were analytical grade or better. All dosing solutions were corrected for salt content and are listed as the dose of free casopitant or ondansetron delivered.

Test Animals, Housing and Dosing

Male ferrets (1-2 kg from Marshall Farms USA, North Rose, NY) were housed in standard conditions with a 12-hour light/dark cycle at Covance Laboratories (Madison, WI). Animals were fed twice daily with non-certified ferret diet and were allowed access to water ad libitum. Since intraperitoneal dosing was used previously to explore the pharmacology of these compounds in the ferret model, these studies were performed using the intraperitoneal route of administration. These studies were approved by the Institutional Animal Care and Use Committee, and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the US National Institutes of Health.
Pharmacokinetic Drug Interaction and Brain Penetration Study

Animal dosing and sampling:
Six ferrets were randomly assigned to 3 groups (Groups 1, 2, and 3), with two animals per group. In dosing session 1, Group 1 received casopitant (0.3 mg/kg, i.p.), Group 2 received ondansetron (0.3 mg/kg, i.p.), and Group 3 received a combination dose of casopitant plus ondansetron (0.3 + 0.3 mg/kg, i.p.). In dosing sessions 2 and 3, animals received an alternate dose regimen in a rotating pattern such that all animals received all three dosing regimens. All dosing solutions were made in sterile saline and reverse osmosis purified water. There was a minimum 48 hour washout period between dosing sessions. In each dosing session, blood samples (0.4 mL) were collected at 0.25, 0.5, 1, 2, 4, 6 and 8 hours post-dosing via a jugular vein vascular access port.

In dosing session 4, the same 6 ferrets were re-assigned to two groups (Groups 4 and 5) with three animals per group. Group 4 received ondansetron (0.3 mg/kg, i.p.) and Group 5 received a combination dose of casopitant plus ondansetron (0.3 + 0.3 mg/kg, i.p.). At 2 hours, blood (0.4 mL) was collected from each animal via a jugular vein vascular access port. Animals were then euthanized (by pentobarbital or isoflurane overdose) and exsanguinated, and the brains excised, rinsed with saline, blotted dry and weighed. Plasma was isolated by centrifugation, and aliquots of blood and plasma were frozen at -70°C. Brain samples were homogenized in sterile water with a 1:1 (v:w, water:brain) ratio using a probe-type homogenizer and frozen at -70°C. All samples were subsequently analyzed for casopitant, the M1 metabolite and/or ondansetron concentrations by LC/MS/MS.

Bioanalytical methods:
For the pharmacokinetic drug interaction study, HTLC/MS/MS methods were developed to quantify ondansetron, casopitant, and the M1 metabolite in ferret plasma, blood and brain tissue homogenate. M1 exists as structural isomers GSK329530/GSK329531 (or the racemic mixture named GSK525060), which were not separated in a short gradient for this experiment. Rather, a GSK329530 calibration curve was used to quantify the total concentration of GSK329531 and GSK329530. All samples were prepared for analysis using protein precipitation with organic solvent followed by HTLC/MS/MS analysis. The HTLC chromatographic system (TLX; Thermo Scientific, Franklin, MA) was coupled to a triple quadrupole mass spectrometer (Sciex API 4000; Applied Biosystems, Foster City, CA). Analytes were ionized by positive atmospheric pressure chemical ionization (APCI). Separation of the analytes from the sample matrix was achieved on a CycloneP TurboFlow column then the analytes were transferred from the TurboFlow column to a Synergi Polar-RP and the analytes eluted from the analytical column with a gradient (60% 10mM Ammonium Formate, pH 3.0 and 40% formic acid in acetonitrile 0.05% by volume, increasing the formic acid in acetonitrile to 95%). The transitions monitored during analysis were $m/z$ 294.0→170.1, 617.5→167.5, and 633.5→183.0 for ondansetron, casopitant, and M1 respectively. The limits of quantification for casopitant, M1 and ondansetron ranged from 1.0 to 5.0 ng/mL.

**Pharmacokinetic Analysis:**

Blood concentration-time data were analyzed by non-compartmental methods using WinNonlin Professional version 4.1 (Pharsight Corp., Mountain View, CA). Area under the curve was calculated using the linear/log trapezoidal method. Because casopitant was rapidly absorbed following intraperitoneal administration, MRT and Vss/F values were not adjusted for drug input.
time. Casopitant, M1 and ondansetron pharmacokinetic parameters were compared using the statistics function in Excel 2002 to compute point estimates for the AUC and Cmax values obtained for each compound when casopitant and ondansetron were administered separately or in combination.

**In Vivo Metabolism and Metabolite Identification Studies**

*Animal dosing and sampling:*

Four male ferrets were administered a single intraperitoneal dose of 6 mg/kg, 5 mL/kg, (specific activity 90 $\mu$Ci/mg) $[^{14}C]$casopitant dissolved in sterile saline. At 2 hours after dosing, 10-20 mL of blood was collected from each animal under anesthesia (1.0-1.5 mL of sodium pentobarbital solution, 65 mg/mL, administered i.p.). Animals were then exsanguinated by upper body cardiovascular perfusion and the brains excised, rinsed with saline, blotted dry and weighed. Upper body perfusion was performed using a chilled solution containing 0.01% v/v heparin and 0.2% v/v sodium nitrite in sterile saline. Buffer was perfused into the left ventricle at the apex of the heart and allowed to flow until the fluid coming from the right ventricle and atrium was clear (total perfusate volume ~0.5 L/kg). Plasma was isolated by centrifugation, and aliquots of blood and plasma were frozen at -70°C. Brain samples were homogenized in sterile water with a 1:1 (v:w, water:brain) ratio using a probe-type homogenizer and frozen at -70°C. Brain homogenate samples were subsequently used for metabolite identification studies.

*Bioanalytical methods:*

For the radiolabel study, an HPLC/MS/MS method was used to quantify $[^{14}C]$casopitant, $[^{14}C]$M2 and $[^{14}C]$M1 (Figure 1) and their respective $[^{12}C]$ analogs in ferret blood, plasma and...
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brain tissue homogenate. Non-radiolabeled analogs were employed as the calibration standards. Chromatographic separation was achieved using a Synergi Polar-RP column (2x100 mm) with a gradient starting at 10mM Ammonium Acetate pH 6.9, 30% Formic Acid in acetonitrile (0.05% by volume) and increasing the organic solvent to 95%. Analytes were ionized by positive TurbolonSpray™ ionization. The transitions monitored for $^{[14}\text{C}]M2$, $^{[14}\text{C}]M1$ and $^{[14}\text{C}]$casopitant were $m/z$ 633.0→181.0, 635.0→165.0, and 619.0→167.0 respectively. The transitions monitored for the $^{[12}\text{C}]$ analogs of M2, M1 and casopitant were $m/z$ 631.4→181.2, 633.0→165.0, and 617.0→167.0 respectively. The limits of quantification for casopitant, M1 and M2 in this assay ranged from 1.0 to 2.5 ng/mL.

Sample preparation and radioanalysis:

Oxidation was performed in a Model 307 Sample Oxidizer (Packard / PerkinElmer, Waltham, MA, USA) and the resulting $^{14}\text{CO}_2$ was trapped in a mixture of Perma Fluor and Carbo-Sorb. Ultima Gold XR scintillation cocktail was used for samples analyzed directly. All samples were analyzed for radioactivity in Model 2900TR liquid scintillation counters (Packard / PerkinElmer, Waltham, MA, USA) for at least 5 minutes or 100,000 counts.

Metabolite identification:

Brain tissue homogenates were extracted with acetonitrile and centrifuged. Extraction recoveries from the resulting supernatants were high, and ranged from 83% to 101%. The profile of radioactivity in the resulting brain homogenate extracts was evaluated by HPLC (Packard 500 Series, Packard / PerkinElmer, Waltham, MA, USA) using a Zorbax RX-C18 column (4.6 x 150 mm) with a gradient of 5 mM ammonium acetate as mobile phase A and acetonitrile:100 mM
ammonium acetate (95:5, v:v) as mobile phase B. The flow rate was 1.2 mL/min. Detection was performed using HP 1100 and 1050 series (Packard / PerkinElmer, Waltham, MA, USA) UV detectors and radioactivity detector (Packard 500 Series, Packard / PerkinElmer, Waltham, MA, USA). Identification of casopitant, M1 and M2 was confirmed by cochromatography with synthetic reference standards.

Mass spectral characterization of casopitant and metabolites M1 and M2:
Positive ion electrospray LC/MS and LC/MS/MS were used to characterize metabolites. Ferret brain extract was analyzed with standard Q1 full-scan function coupled with radiomatic detection. Enhanced Product Ion (EPI) experiment was performed on m/z 619.4, 635.4 and 633.5 ions using Profile scan mode with a scan rate of 4000 amu/second and a scan range of 100 - 625, 80 - 650 and 100 - 640 amu respectively. After the mass-to-charge ratio (m/z) of the molecular ion was determined for casopitant and metabolites M1 and M2 at their respective retention times based on the full-scan and radiomatic data, the samples were re-analyzed using EPI scan of the m/z ratio. EPIs of casopitant [~26 minutes], metabolite M1 [~25 minutes] and metabolite M2 [~39 minutes] were performed for comparison with reference standards.

LC/MS instrumentation and software for the characterization of casopitant and metabolites M1 and M2:
LC was performed on a Shimadzu pump (model LC-10AD VP), equipped with an injector (Model SIL-HTc), a column oven (Model-10AC VP (25 °C), a valve switch (model CTO-10AC VP) and Agilant HPLC column (Rx-C18 5 um, 50 mm x 4.6 mm i.d.). A gradient was performed using 5 mM ammonium acetate as mobile phase A and acetonitrile:100 mM
ammonium acetate (95:5, v:v) as mobile phase B (flow rate of 1.2 mL/min). Mass spectrometric analysis was performed using Applied Biosystems 4000 Q Trap with Turbo Spray source, equipped with Analyst 1.4 and Metabolite ID 1.4 software (Applied Biosystems, Foster City, CA, USA). Radiochemical detection was performed using a Radiomatic Series 500 detector (Packard / PerkinElmer, Waltham, MA, USA) equipped with Flo-One Version 3.65 software (Packard / PerkinElmer, Waltham, MA, USA). Scintillation flow rate was 1.8 mL/min (Ultima-Flo M LSC cocktail, Packard / PerkinElmer, Waltham, MA, USA).

After passing through the column switcher, the HPLC column effluent was split with approximately 50% of the flow diverted to the mass spectrometer and 50% to the radiometric detector. The Q1 full scan mass spectrometer analysis was performed using Turbo Spray positive ionization mode with Ion Spray Voltage of 3000V and a Scan time of 0 to 80 minutes. The source temperature was 500°C.

**In vitro receptor binding affinity of casopitant and metabolites to ferret and human NK-1 receptor**

Competition binding assays were performed using ferret membrane homogenates (60 to 75 µg of protein) with [125I]-tyr8-substance P at 100 pM, the Kd concentration at ferret NK-1 receptors as described previously (Rissler et al, 1997). Nonspecific binding for each NK-1 receptor source was defined in the presence of 0.5 µM GR205171. Assays were terminated by rapid filtration onto 0.3% polyethylenamine-treated GF/C filters (Inotech Biosystems International, Rockville, MD, USA), and bound radioactivity (cpm) was quantified using a TopCount scintillation counter (PerkinElmer Life Sciences, Waltham, MA, USA).
RESULTS:

Pharmacokinetic Drug Interaction and Brain Penetration Study

Following single intraperitoneal dose administration of 0.3 mg/kg, casopitant was rapidly absorbed, with Tmax values occurring within 1 hour (Table 1). Mean CL/F and Vss/F values were 49.9 mL/min/kg and 11.5 L/kg, respectively, following administration of casopitant alone, and 48.7 mL/min/kg and 13.7 L/kg following administration of the combination dose (Table 1). Concentrations of casopitant and the M1 metabolite were approximately equal in blood and plasma, resulting in an M1-to-casopitant plasma concentration ratio that was similar to unity. The brain-to-blood concentration ratios for casopitant were 0.97±0.78, and for M1 were 0.26±0.20. Ondansetron was not quantifiable in brain homogenate samples.

The point estimates of Cmax and AUC for casopitant, the M1 metabolite, and ondansetron obtained when casopitant and ondansetron were dosed alone or in combination were similar to one, with all mean point estimates being within one standard deviation of unity (Table 2). Concomitant administration of casopitant and ondansetron appeared to have no effect on the disposition of either agent, or on the concentrations of the M1 metabolite of casopitant.

In Vivo Metabolism and Metabolite Identification Studies

HPLC Profiling:

Three radioactive peaks were identified in brain homogenate extracts and confirmed through cochromatography with reference standards. The major radioactive component in brain samples
was parent compound, which accounted for approximately 76% of the radioactivity (Figure 2, Table 3). Two metabolites present in brain tissue following administration of [14C]casopitant were hydroxylated casopitant (M1), and the ketone product of the M1 metabolite (M2), which accounted for approximately 19% and 3% of the radioactivity in the brain extracts, respectively. The brain-to-blood concentrations ratios for casopitant, M1 and M2 in this study were 0.955, 0.395 and 1.090, respectively (Table 4).

Metabolite M1 was identified as hydroxylated casopitant, based on mass spectral analysis and cochromatography with reference standard GSK329530. Compared with the radioactive signal, the mass-to-charge ratio (m/z) of the metabolite M2 at 41.9 minutes was determined to be 633.5 from the Q1 full-scan data. Metabolite M2 was proposed to be a dehydrogenated product (ketoisomer) of metabolite M1. The structure of metabolite M2 was confirmed by comparison to the reference standard GSK1497960A. Enhanced product ion mass spectra of reference standards for casopitant, M1, and M2, and the corresponding spectra from ferret brain extract, are presented in Figures 3, 4, and 5, respectively. It should be noted that certain ions in the spectra obtained from the ferret brain extracts are 2 Da higher than the corresponding ions in the reference standards, due to the presence of the [14C]-label in the samples from ferret brain. Some of the major mass spectral fragmentations for the parent and the metabolites M1 and M2 are also shown in the inset structures in the mass spectra figures.

Additional characterization of the parent and metabolites M1 and M2 was derived from the quantitative mass spectral analysis of the pooled extract of brain homogenate, blood and plasma samples obtained following administration of [14C]casopitant. Quantitative analysis was
performed using triple quadrupole mass spectrometer by the methods described above employing non-radiolabeled standards. The specific MRM transitions used for the analysis of parent, metabolite M1 and metabolite M2 in these metrics were, m/z 619→167, 635→165 and 633→181 respectively. This analysis provides additional confirmatory proof for the presence of parent, metabolite M1 and M2 in the ferret brain extract.

**In vitro receptor binding affinity of casopitant and metabolites to ferret and human NK-1 receptor**

Casopitant and metabolites M1 and M2 all had high affinity for ferret NK-1 receptors endogenously expressed in ferret brain cortical membranes (Ki < 1 nM, Table 5).

**DISCUSSION:**

There is strong evidence in the literature to suggest that the beneficial effects of NK-1 receptor antagonists on chemotherapy-induced nausea and vomiting are dependent upon brain penetration and subsequent binding to receptors in the brain (King, 1990; Huskey et al, 2003). The pharmacokinetics and brain concentrations of a novel NK-1 receptor antagonist, casopitant, were therefore examined in ferrets, a species previously used to assess the therapeutic potential of this drug as a treatment for nausea and vomiting following chemotherapy. Blood, plasma and brain tissue were examined following single intraperitoneal administration of casopitant or [14C]casopitant. The intraperitoneal route of administration was previously chosen over the oral route for pharmacodynamic studies in order to eliminate potential artifacts due to mechanical reflex induced vomiting observed with usage of oral dosing gavage tubes in ferret emesis models. The pharmacokinetic drug interaction study was conducted at a dose (0.3 mg/kg)
similar to that shown to provide anti-emetic activity in acute and delayed emesis models in the ferret (King, 2006). Higher doses were chosen for the brain penetration and in vivo metabolite identification study to ensure that brain tissue concentrations would be quantifiable. As was expected, significant concentrations of casopitant were detected in both brain and blood samples following single dose administration of the 6 mg/kg dose. Although the half-life of casopitant was found to be relatively short, with values ranging from 3-5 hours in the ferret, casopitant has been previously shown to provide complete protection from emetic events for 6 hours following a single dose administration in a cisplatin-induced acute emesis model in the ferret (King, 2006). This same dose also provides partial protection for 72 hours after a single dose in the delayed emesis model. We therefore examined whether metabolites of casopitant were contributing to this prolonged duration of action.

Several metabolites of casopitant had been identified previously following incubation in liver microsomes or hepatocytes from various species. However, it was not known if the major metabolites of casopitant have NK-1 antagonist activity, or if the prolonged pharmacodynamic effects of casopitant observed in the ferret were due to casopitant itself, one of its metabolites, or a combination. Since pharmacological effect is presumed to be associated with brain penetration, plasma, blood and brain tissue were collected from ferrets dosed with \[^{14}\text{C}]\text{casopitant. Two hours following a single dose of casopitant, the major radioactive component in brain tissue was observed to be parent drug. In addition to a known circulating human hydroxylated metabolite of casopitant (M1), a novel metabolite, the corresponding ketone product of the M1 metabolite (M2) was identified and its structure confirmed by comparison to a synthesized reference standard. The brain-to-blood concentration ratios for both casopitant and}
M2 were approximately one, suggesting that both compounds penetrated readily into brain tissue. However, concentrations of casopitant in both blood and brain were approximately 25-fold higher than concentrations of M2. The brain-to-blood concentration ratio for M1 was approximately 0.4, suggesting poorer penetration of this metabolite into the brain. Although no prior validation of the upper body perfusion method used in the metabolism study was performed to determine if compound would be lost from brain tissues into the perfusate buffer, the casopitant brain-to-blood concentration ratios obtained in the metabolism study following upper body perfusion and in the drug interaction study (no perfusion) were comparable, suggesting that there was minimal loss of drug from brain tissues following perfusion.

Since NK-1 receptor antagonists have previously been shown to provide incremental improvement in efficacy when used in combination with 5-HT3 antagonists, casopitant has been administered concomitantly with ondansetron in the ferret model of chemotherapy-induced nausea and vomiting, yielding therapeutically synergistic effects (Gagnon and King, 2006). Because of this pharmacodynamic synergy, the potential pharmacokinetic drug-drug interaction of casopitant and ondansetron was investigated in a three-way crossover study. The point estimates of Cmax and AUC for casopitant, the M1 metabolite, and ondansetron obtained when casopitant and ondansetron were each dosed alone or in combination were similar to one, indicating that concomitant administration of casopitant and ondansetron had no effect on the disposition of either agent, or on the concentrations of the M1 metabolite. The brain-to-plasma concentration ratios for casopitant and the M1 metabolite following concomitant administration of casopitant and ondansetron were similar to those observed following administration of casopitant alone, suggesting that coadministration of casopitant with ondansetron did not alter
brain penetration of casopitant or its M1 metabolite. Concentrations of the M2 metabolite were not measured in this study, but they are expected to have been very low.

In humans, casopitant has been shown to be an efficacious therapy in patients with chemotherapy-induced nausea and vomiting (Arpornwirat et al, 2006; Rolski et al, 2006). The in vitro binding affinity of casopitant to ferret brain cortical NK-1 appears to be slightly greater compared to its M1 and M2 metabolites (Table 5), although the potency of the three molecules is not markedly different. The affinities of casopitant, M1 and M2 at human NK-1 receptors expressed on CHO cell membranes were found to be similar to those determined for ferret brain NK-1 receptors (data not shown), suggesting comparable potency at the site of action in the two species. Previous biochemical affinity studies demonstrated equivalent binding pharmacology at both human and ferret receptors for known NK-1 antagonists (Tattersall et al, 1996). In addition, the relative concentrations of M1 and M2 in the ferret brain were observed to be much lower than the concentrations of casopitant (Table 4). Given the relatively similar potency and selectivity of casopitant and its hydroxyl and ketone metabolites, the pharmacologic activity of casopitant in the ferret can be largely attributed to parent compound and to a lesser extent, its hydroxylated and ketone metabolites. Substantial brain concentrations of casopitant, M1 and M2 in excess of their respective in vitro IC50 values for protracted periods may explain the prolonged duration of action.
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REFERENCES:


FOOTNOTES

Citation of abstracts previously presented:


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FIGURE LEGENDS

Figure 1. Structures of casopitant and its metabolites.

Figure 2. Representative radioactivity profile of casopitant and its metabolites in pooled brain homogenate extract following i.p. dosing with \(^{14}C\)casopitant at 6 mg/kg. At 120 minutes postdose, brain extracts from ferrets were subjected to HPLC analysis. Chromatography was performed using a Zorbax RX-C18 column as described in Materials and Methods.

Figure 3. Enhanced product ion spectra of casopitant reference standard (upper panel) and \(^{14}C\)casopitant in ferret brain extract (lower panel). Positive ion electrospray LC/MS and LC/MS/MS were used to analyze casopitant in brain extract samples and reference standards.

Figure 4. Enhanced product ion spectra of metabolite reference standard M1 (upper panel) and M1 metabolite in ferret brain extract (lower panel). Positive ion electrospray LC/MS and LC/MS/MS were used to analyze M1 metabolite in brain extract samples and reference standards.

Figure 5. Enhanced product ion spectra of metabolite reference standard M2 (upper panel) and M2 metabolite in ferret brain extract (lower panel). Positive ion electrospray LC/MS and LC/MS/MS were used to analyze M2 metabolite in brain extract samples and reference standards.
LC/MS and LC/MS/MS were used to analyze M2 metabolite in brain extract samples and reference standards.
Table 1. Pharmacokinetic parameter estimates obtained for casopitant, M1 and ondansetron following administration of casopitant and ondansetron separately or in combination. Values presented are the mean ± SD of n= 6 animals per dosing regimen.

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<td>Vss/F (L/kg)</td>
<td>11.5 ± 4.7</td>
<td>13.7 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>M1 metabolite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.17 ± 0.98</td>
<td>1.83 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>21.10 ± 5.44</td>
<td>23.17 ± 7.08</td>
<td></td>
</tr>
<tr>
<td>AUC(0-t) (ng*h/mL)</td>
<td>116.43 ± 27.64</td>
<td>125.75 ± 39.80</td>
<td></td>
</tr>
<tr>
<td>Ondansetron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>0.29 ± 0.02</td>
<td>0.30 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.25 ± 0.00</td>
<td>0.25 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>64.80 ± 32.82</td>
<td>73.72 ± 14.16</td>
<td></td>
</tr>
<tr>
<td>AUC(0-t) (ng*h/mL)</td>
<td>34.38 ± 20.98</td>
<td>41.74 ± 5.70</td>
<td></td>
</tr>
<tr>
<td>AUC(0-inf) (ng*h/mL)</td>
<td>36.59 ± 20.15</td>
<td>43.48 ± 4.79</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Point estimates for AUC and Cmax obtained for casopitant, M1 and ondansetron following administration of casopitant and ondansetron separately or in combination. Values presented are the mean ± SD of n= 6 animals per dosing regimen.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Point Estimate</th>
<th>Point Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax</td>
<td>AUC</td>
</tr>
<tr>
<td>casopitant</td>
<td>0.95 ± 0.39</td>
<td>1.09 ± 0.57</td>
</tr>
<tr>
<td>M1</td>
<td>0.97 ± 0.36</td>
<td>1.01 ± 0.44</td>
</tr>
<tr>
<td>ondansetron</td>
<td>1.30 ± 0.46</td>
<td>1.47 ± 0.63</td>
</tr>
</tbody>
</table>
Table 3. Representative retention times and percent activity of casopitant and its metabolites in pooled brain homogenate extract following i.p. dosing with [14C]casopitant at 6 mg/kg (at 120 minutes postdose, brain extracts from ferrets were subjected to HPLC analysis).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention Time (minutes)</th>
<th>Percent Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>28.2</td>
<td>19.25</td>
</tr>
<tr>
<td>casopitant</td>
<td>30.5</td>
<td>76.13</td>
</tr>
<tr>
<td>M2</td>
<td>41.9</td>
<td>3.03</td>
</tr>
</tbody>
</table>
Table 4. Concentrations of [14C]casopitant and its metabolites in ferret brain following a single intraperitoneal dose of 6 mg/kg. Values presented are the mean of n= 2 animals per analyte.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Brain Concentration (ng/g tissue, µM)</th>
<th>Brain:Blood Concentration Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casopitant</td>
<td>499 (0.809)</td>
<td>0.955</td>
</tr>
<tr>
<td>M1</td>
<td>149 (0.235)</td>
<td>0.395</td>
</tr>
<tr>
<td>M2</td>
<td>19.7 (0.031)</td>
<td>1.090</td>
</tr>
</tbody>
</table>
Table 5. Binding affinities of casopitant and its metabolites in ferret brain cortical NK-1 receptor binding assays. Values presented are the mean ± SD (n=8).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki ± SD (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ferret Brain Cortical NK-1</strong></td>
<td></td>
</tr>
<tr>
<td>Casopitant</td>
<td>163 ± 39</td>
</tr>
<tr>
<td>M1</td>
<td>464 ± 132</td>
</tr>
<tr>
<td>M2</td>
<td>108 ± 31</td>
</tr>
</tbody>
</table>
Figure 1

Casopitant

M1

M2

* Denotes the location of $^{14}$C label
Figure 3

[Chemical structure with m/z values]

- $[M + H]^+ = 617$
- $346 - C_3H_4NO = 277$
- $167 - CH_3CO. = 124$

Relative Intensity (%) vs. m/z, amu