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In vitro and *in vivo* characterization of amorphous, nanocrystalline, and crystalline ziprasidone formulations

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ABSTRACT

Ziprasidone, commercially available as Geodon® capsules, is an atypical antipsychotic used in the treatment of schizophrenia and bipolar disorder. It is a BCS Class II drug that shows up to a 2-fold increase in absorption in the presence of food. Because compliance is a major issue in this patient population, we developed and characterized solubilized formulations of ziprasidone in an effort to improve absorption in the fasted state, thereby resulting in a reduced food effect. Three formulations utilizing solubilization technologies were studied: (1) an amorphous inclusion complex of ziprasidone mesylate and a cyclodextrin, (2) a nanosuspension of crystalline ziprasidone free base, and (3) jet-milled ziprasidone HCl coated crystals made by spray drying (CCSD) the drug with hypromellose acetate succinate. The formulations were characterized by *in vitro* methods appropriate to each particular solubilization technology. These studies confirmed that ziprasidone mesylate – cyclodextrin was an amorphous inclusion complex with enhanced dissolution rates. The ziprasidone free base crystalline nanosuspension showed a mean particle size of 274 nm and a monomodal particle size distribution. In a membrane permeation test, the CCSD showed a 1.5-fold higher initial flux compared to crystalline ziprasidone HCl. The three formulations were administered to fasted beagle dogs and their pharmacokinetics compared to Geodon® capsules administered in the fed state. The amorphous complex and the nanosuspension showed increased absorption in the fasted state, indicating that solubilized formulations of ziprasidone have the potential to reduce the food effect in humans.

1. Introduction

Ziprasidone (Fig. 1) is a chlorooxyindole class aryl-heterocyclic that is an atypical anti-psychotic agent used in the acute and long-term treatment of schizophrenia and manic symptoms of bipolar disorder (Geodon®, 2009). These are highly debilitating mental disorders – more than 5.7 million Americans suffer from bipolar disorder and 2.4 million Americans suffer from schizophrenia in a year. Ziprasidone binds to a variety of receptors (D_2, D_3, $5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_{1D}$ and α_1) with low nanomolar affinity. Although its exact mechanism of action is unknown, it is believed to be related to D_2 and $5HT_{2A}$ antagonism (Stahl and Shayegan, 2003). Ziprasidone was first approved in the U.S. in February 2001. The commercially available ziprasidone formulations, which include an oral capsule, an oral suspension, and an immediate-release intramuscular formulation, are marketed worldwide under the trade names of Geodon® or Zeldox®. Over the years, the safety (Daniel, 2003; Patel and Keck, 2006; Zimbroff Dan et al., 2005), efficacy (Greenberg and Citrome, 2007; Harrison and Scott, 2006; Patel and Keck, 2006; Zimbroff Dan et al., 2005), and cost-effectiveness (Bernardo et al., 2006, 2007; Bobes et al., 2004) of ziprasidone have been well established.

Ziprasidone shows up to a 2-fold increase in the rate (*i.e.*, C_{max}) and extent (*i.e.*, AUC_{inf}) of absorption in the presence of a high calorie-high fat meal (Miceli et al., 2000). Studies have shown that calories consumed (rather than the fat content of the meal) and the time between dosing and food intake are important factors in the absorption of ziprasidone (Gandelman et al., 2009; Hamelin et al., 1998; Lincoln et al., 2010). When dosed orally with food, Geodon® exhibits linear pharmacokinetics in the 20–80 mg dose range. Because of this food effect, it is recommended that Geodon® capsules be taken with food (Geodon®, 2009). However, compliance can be a major issue in patients with schizophrenia; it is estimated that around 50% of the patients do not fully comply with the prescribed treatment (Perkins, 2002). Thus, a ziprasidone formulation with no food effect has the potential to enhance

Abbreviations: AUC_{inf}, area under the serum concentration–time profile from time 0 extrapolated to infinite time; AUC_{last}, area under the serum concentration–time profile from time 0 to the time of the last quantifiable concentration (C_{last}); C_{max}, maximum serum concentration; T_{max}, time for C_{max}.

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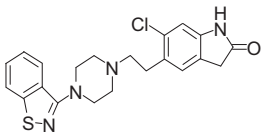

Fig. 1. Chemical structure of ziprasidone.

the effectiveness of this medication in a poorly compliant patient population.

Several review articles have examined food–drug interactions (Melander, 1978; Schmidt and Dalhoff, 2002; Singh, 1999; Toothaker and Welling, 1980; Welling, 1996). Increased oral bioavailability when dosed with food has been attributed to various factors such as micellar solubilization due to bile acids, delayed stomach emptying, decreased hepatic clearance, and increased bile flow leading to reduced presystemic and first pass metabolism (Singh, 1999; Singh and Malhotra, 2004). Ziprasidone HCl is a weak base with a high melting point (304 °C), high lipophilicity ($c\log P = 3.6$), and an intrinsic solubility of 0.3 μg/mL (Friesen et al., 2008; Kim et al., 1998). Based on a partition coefficient into bile salt micelles of about 2200, its solubility in simulated biorelevant fluids was estimated to be 4–5 μg/mL in fasted state media and 10–14 μg/mL in fed state media. We hypothesized that ziprasidone shows solubility and/or dissolution rate-limited absorption in the fasted state, so we attempted to improve the extent of absorption through the use of various solubilization technologies.

Numerous technologies are available for improving the solubility or dissolution rates of a drug, including prodrugs (Fleisher et al., 1996; Stella and Nti-Addae, 2007), polymorphs, solvates, co-crystals (Babu and Nangia, 2011), salt formation (Serajuddin, 2007), lipid based systems (Hauss, 2007; Humberstone and Charman, 1997; Porter et al., 2008), micellar solubilization including self-emulsifying drug delivery systems (Tang et al., 2007), inclusion complexes (Brewster and Loftsson, 2007), amorphous solid forms such as spray dried dispersions (Friesen et al., 2008), and particle size reduction including nanomilling (Kesisoglou et al., 2007; Merisko-Liversidge et al., 2003; Usha et al., 2010).

In this study, we developed and characterized the following solubilized forms of ziprasidone in an attempt to reduce or eliminate its food effect: an amorphous ziprasidone mesylate – cyclodextrin complex, a suspension containing nanocrystalline ziprasidone free base, and an intimate physical mixture of a jet-milled ziprasidone hydrochloride with a polymeric precipitation inhibitor.

2. Methods and materials

2.1. Materials

Ziprasidone free base, mesylate, and hydrochloride salts were obtained from Pfizer Inc. Sulfobutyl ether β-cyclodextrin sodium (SBECD) was manufactured by Pfizer Inc. under license purchased from CyDex Pharmaceuticals, Inc. (Lenexa, KS, USA). Hypromellose acetate succinate AQOAT®, HG-grade, referred to in this paper by its older name, hydroxypropyl methylcellulose acetate succinate (HPMCAS), was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Poloxamer 338 (Pluronic® F108) was purchased from BASF Corp. (Mount Olive, NJ, USA). Polysorbate 80 (Tween® 80) and Soybean lecithin (NF grade) were purchased from Spectrum Chemicals (Gardena, CA, USA). All other chemicals, reagents, and solvents were analytical grade and were purchased from commercial suppliers by Pfizer Inc.

Table 1
Summary of solubilized ziprasidone formulations investigated in this study.

Formulation	Acronym	Brief Description
A	Ziprasidone–SBECD complex	Ziprasidone mesylate – sulfobutyl ether β-cyclodextrin inclusion complex
B	Ziprasidone nanosuspension	Ziprasidone free base nanosuspension made by wet-milling
C	Ziprasidone CCSD	Jet-milled ziprasidone HCl crystals coated with hydroxypropyl methylcellulose acetate succinate HG-grade (HPMCAS) as a precipitation inhibitor

2.2. Test formulations and in vitro characterization

Three solubilized formulations, designated as formulations A, B, and C in Table 1, were made and characterized as described below.

2.2.1. Test formulation A

A ziprasidone mesylate – SBECD inclusion complex was prepared by first making an aqueous solution of ziprasidone mesylate and SBECD at a molar ratio of 1:1.3 in a stirred vessel maintained at 75 °C. The solution was then cooled to 40 °C and filtered, under nitrogen, through a 0.45 μm Kleenpak Ultipor N66 filter (Pall Corporation, Port Washington, NY, USA). The filtered solution was dispensed into stainless steel drying trays, lyophilized at −32 °C for at least 96 h, dried at −12 °C until there was a break in the product cake, followed by drying at 25° for 24 h. The dried cake was milled to a powder using a Fitzpatrick M5A mill (The Fitzpatrick Co., Elmhurst, IL, USA) fitted with a 0.0315 in. rasping plate and bar impeller rotating at 1020 rpm.

The stoichiometry of ziprasidone mesylate-SBECD inclusion complex is 1:1 (Kim et al., 1998). A slight excess of SBECD relative to ziprasidone mesylate was selected to ensure that the drug was completely complexed without significantly decreasing the drug loading, which would have resulted in a dosage form of a large size. A crystalline ziprasidone mesylate-SBECD physical mixture was prepared at the same molar ratio of 1:1.3 to serve as a control.

Formulation A and the physical mixture were characterized by light microscopy, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and SS-NMR to determine the form of the drug in the solid state. In addition, dissolution studies were performed at pH 4.0 and pH 7.4.

2.2.1.1. Light microscopy. Microscopic analyses of ziprasidone mesylate, SBECD, and formulation A were performed using an Olympus BH-2 microscope under bright and dark field.

2.2.1.2. Powder X-ray diffraction (PXRD). PXRD patterns of ziprasidone mesylate, SBECD, a physical mixture of ziprasidone with SBECD, and formulation A were obtained on a D5000 – Siemens Diffractometer with a voltage of 50 kV and a current of 40 mA. Alignment was verified with an aluminum standard before each measurement. Samples were prepared by placing powders in a quartz zero background sample holder and scanned from 3° to 40° 2θ at a rate of 1° per second.

2.2.1.3. Solid-state nuclear magnetic resonance (SS-NMR). Inclusion complexation of ziprasidone with SBECD was confirmed using proton NMR of ziprasidone solubilized in SBECD (Kim et al., 1998). SS-NMR was also used to characterize whether a molecular level interaction between SBECD and ziprasidone was maintained in the solid state lyophilized complex. The details of the methodology and results have been described previously (Hong et al., 2011).

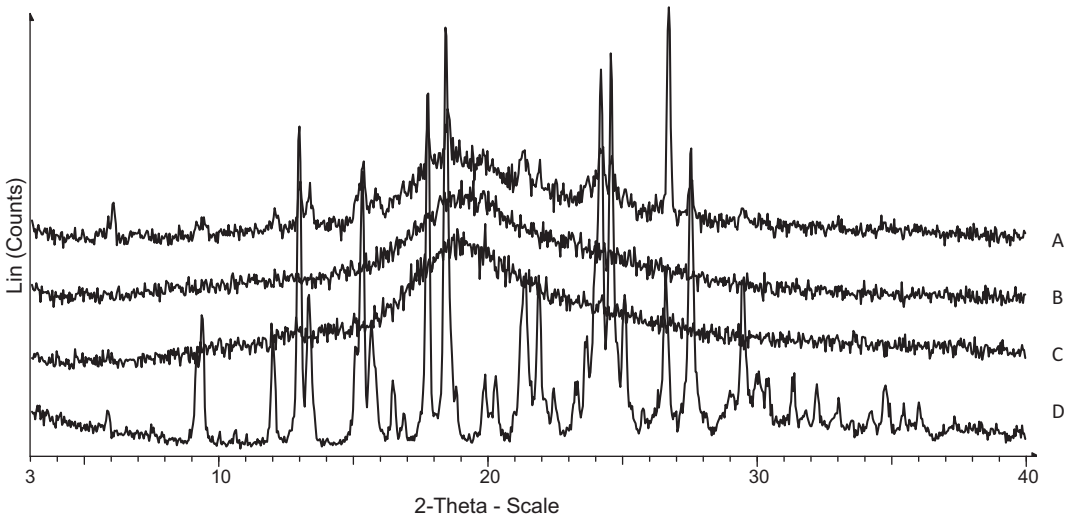

Fig. 2. Powder X-ray diffraction patterns of (A) physical mixture of ziprasidone mesylate and SBECD, (B) SBECD alone, (C) formulation A, and (D) crystalline ziprasidone mesylate.

2.2.1.4. Dissolution at pH 4.0 and pH 7.4. Dissolution studies were performed with formulation A and the physical mixture of ziprasidone mesylate and SBECD of an identical composition (same molar ratio) at pH 4.0 and pH 7.4 using 50 mM phosphate buffer. An amount of powder equivalent to 20 mg of ziprasidone was added to 200 mL of the dissolution medium in a beaker with stirring. At various time points, a 1 mL aliquot of the dissolution medium was withdrawn, filtered through a 0.22 μm Milipak® syringe filter (Millipore Corporation, Billerica, MA, USA) into a vial, and analyzed by reversed-phase HPLC. The chromatographic conditions were: Zorbax Rx C8 column (Agilent Technologies, Santa Clara, CA, USA); 40 °C; mobile phase – 0.05 M KH_2PO_4 pH 6.5/acetonitrile (55/45 v/v); flow rate – 1.0 mL/min; injection volume – 10 μL; UV detection at λ = 315 nm). A 1 mL aliquot of fresh dissolution media was added back to the beaker to replace the sample volume. Ziprasidone is a weak base with a pK_a of about 6.5 and solubility of the free base of about 0.3 μg/mL (Kim et al., 1998). Therefore, while dissolution at pH 4.0 was under sink conditions, the dissolution at pH 7.4 was under non-sink conditions.

2.2.2. Test formulation B

A 210 mg/mL ziprasidone free base nanosuspension was first prepared as follows: a coarse suspension was prepared by placing ziprasidone free base in the 100 mL milling chamber of a Nanomill-1™ [Manufacturer: Elan Drug Delivery, Inc., King of Prussia, PA] along with the grinding media consisting of 500 μm size polystyrene beads. Poloxamer 338 (Pluronic® F108), Polysorbate 80 (Tween® 80), and Soybean Lecithin (NF grade), used as surface stabilizers/surface modifiers, were then added to the milling chamber either as an aqueous solution (poloxamer and polysorbate) or as an aqueous dispersion (lecithin). The suspension was then milled for 80 min at 2100 rpm and the temperature during milling was maintained at 4 °C. The resulting suspension was filtered under vacuum to remove the milling media (Shah et al., 2008). The concentrations of individual components in the nanosuspension were: ziprasidone free base – 210 mg/mL, poloxamer 338 and polysorbate 80 – 10 mg/mL each, and lecithin – 5 mg/mL. The nanosuspension was characterized for particle size distribution and physical stability.

2.2.2.1. Particle size measurement. The particle size of formulation B was measured by light diffraction with a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). After acquiring background information, formulation B was added drop-wise to the dilution chamber containing water, and particle sizing was started immediately. The resulting dilution was approximately 1000-fold and the resulting light obscuration was 5–10%. Particle sizing was repeated as necessary at various time points to ensure physical stability of formulation B during measurement.

2.2.2.2. Powder X-ray diffraction (PXRD). PXRD patterns of formulation B and a sample of ziprasidone free base were obtained by the method outlined in Section 2.2.1.2.

2.2.2.3. Stability of nanosuspension. Formulation B was filled into vials and stored at 5 °C. Samples at various time intervals were analyzed for ziprasidone potency, purity profile, and particle size, as described above, to assess physical stability.

2.2.3. Test formulation C

Ziprasidone HCl bulk powder was milled using a Laboratory Jet-Mill (Glen Mills, Inc., Clifton, NJ, USA). The jet-milled crystals were suspended in a 10 wt% solution of HPMCAS-HG (35%, w/w ziprasidone active relative to HPMCAS-HG) in acetone (final solids content in acetone = 10 wt%) and spray dried using a Niro PSD-1 Spray Dryer (GEA Process Engineering, Inc., Columbia, MD, USA) with a SK 74-20 nozzle (Spraying Systems Co., Whaton, IL, USA). The spray drying parameters were: drying gas (nitrogen) flow rate 1550 g/min; inlet temperature 140 °C; outlet temperature 40 °C; feed pressure 350 psi; and suspension feed rate 285 g/min. The solubility of ziprasidone HCl in acetone was 0.002%, making it unlikely that it was dissolved and then immediately re-crystallized out during or after spray drying.

2.2.3.1. Microscopy, powder X-ray diffraction and particle size. Formulation C was characterized using a Hitachi S-3400 scanning electron microscope (SEM) (Hitachi High Technologies, Schaumburg, Illinois). Samples were examined using a Bruker D8 Advance Powder X-ray diffractometer (Madison, Wisconsin), and the particle size was measured using a Malvern Mastersizer Sirocco 2000 (Malvern Instruments, Malvern, UK).

2.2.3.2. Membrane permeation test. An *in vitro* membrane permeation test of formulation C was performed as follows. A 5 mL feed solution of the CCSD was prepared in pH 6.5 phosphate buffer with 0.5% sodium taurocholic acid/1-palmitoyl-2-oleoyl-sn-3-glycerophosphatidylcholine (NaTC/POPC) and placed in contact

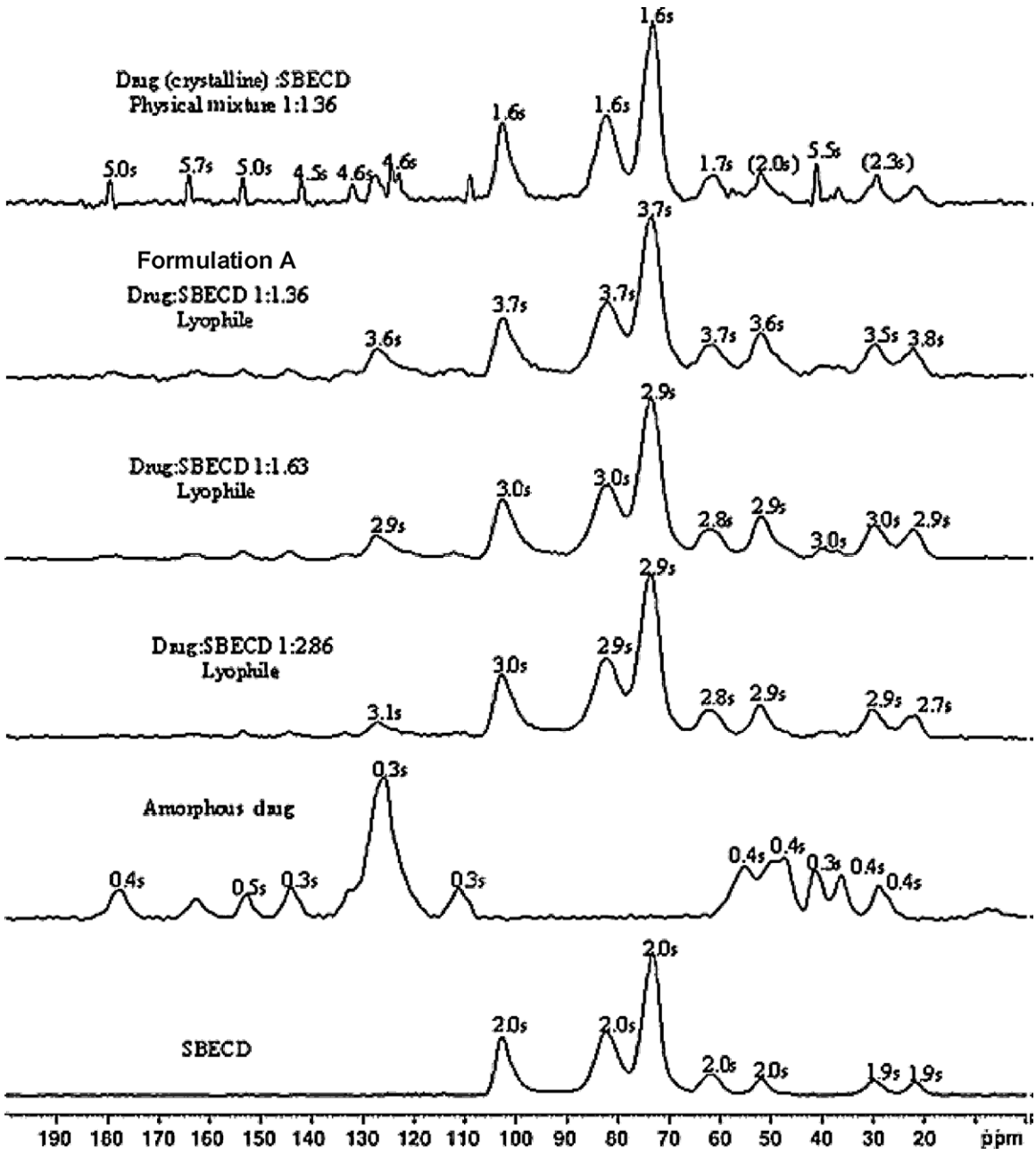

Fig. 3. Solid state NMR ^{1}H T_1 relaxation study for ziprasidone.

Reproduced from Hong et al. (2011).

with the plasma treated (*i.e.*, hydrophilic) side of a microporous polypropylene membrane (Accurel®; pore size = 0.1 μm; thickness = 90 μm, Membrana GmbH, Wuppertal, Germany). The other (less hydrophilic) side of the membrane was in contact with 5 mL of the permeate solution, which consisted of 60% decanol:40% decane. The permeate solvent mixture provided a sink for the drug in the permeate relative to the aqueous media of interest. The effective membrane area on the permeate side was about 4.9 cm^2. The rate of transport of the drug from the feed solution to the permeate solution was determined at 37 °C by taking 50 μL samples from the permeate solution and measuring the concentration of drug as a function of time using an HPLC-UV assay. The HPLC assay utilized a normal phase HPLC method using a Zorbax SB-CN, 4.6 mm × 150 mm, 5 μm column, a 1.2 mL/min flow rate, a 50 μm injection volume, a 25 °C column temperature, 230 nm UV detection, and integrating the peak at 2.6 min retention time. The diluent was isopropyl alcohol, and the mobile phase was 16.3/83.7 (60/40 decanol/decane)/IPA. The maximum flux of drug across the membrane was determined by performing a least squares fit to the data to obtain the slope, multiplying by the permeate volume, and dividing by the membrane area.

2.3. Pharmacokinetic studies in dogs

The *in vivo* pharmacokinetic study described here was reviewed and approved by the Pfizer Groton Institutional Animal Care and Use Committee and adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985). The objective of the study was to characterize the pharmacokinetics of solubilized formulations of ziprasidone in six fasted male beagle dogs compared to the ziprasidone formulation in the commercial Geodon® capsule (reference) dosed in the fed state. Crystalline ziprasidone

monohydrochloride monohydrate is present in Geodon® capsules. The dogs were administered with the reference (20 mg dose) or one of the test formulations (40 mg dose) in a crossover manner with at least a one week washout period. The test formulations were: formulation A: ziprasidone mesylate-SBECD complex dosed in two size #0 capsules; formulation B: ziprasidone free base nanosuspension dosed as a diluted suspension in 60 mL water by oral gavage; formulation C: jet-milled ziprasidone hydrochloride coated crystals by spray-drying (CCSD) dosed in one size #0 capsule. The test formulations were dosed at 40 mg *versus* 20 mg of the reference formulation to ensure that ziprasidone serum levels were not under the limit of quantification in case any of the test formulations did not produce enhanced absorption in the fasted state.

In the fasted studies, on the day prior to the study, each dog received two 8 oz. cans of Rebound® Liquid Diet (Virbac AH, Inc., Fort Worth, TX) in order to satisfy caloric requirements. After an overnight fast, they were each dosed with the test formulation followed by an oral gavage of 50 mL water. In the case of test formulation B, a volume of the suspension corresponding to a 40 mg dose was diluted in 60 mL of water and administered as a suspension followed by 60 mL of water rinse. In the fed studies, 15 min prior to dosing, each dog was fed 10 g of High Density Canine Diet 5L18 and then dosed with the reference formulation followed by an oral gavage of 50 mL water. Various amounts of food were tested to induce a fed state in dogs for ziprasidone to reproduce the 2-fold food effect seen in humans (unpublished data), and 10 g of dry dog food was established as appropriate to study the food effect of the test formulations. After administration of the dose, the dogs were returned to metabolism cages with free access to water. They were fed their normal rations after the 8 h sampling time point.

Whole blood samples of ~4 mL were taken from the jugular vein using 5 cc red Vacutainers® with 21 gauge needles at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 h post-dose. The samples were centrifuged for 10 min at 3000 rpm in a 5 °C centrifuge. The resultant serum were transferred to 2 mL cryogenic vials and frozen at −10 °C until assayed. The samples were extracted using a published procedure (Janiszewski et al., 1995). Analysis was performed using a PerkinElmer-SCIEX API III HPLC/MS/MS system. Chromatography was performed on a Waters Atlantis C18, 2.1 mm × 50 mm, 5 μm with an isocratic elution using 62% solvent A (acetonitrile with 0.05% formic acid) and 38% solvent B (10 mM ammonium acetate with 0.05% formic acid) at 0.3 mL/min. The ion transitions monitored were m/z 413.2 → 193.94 for ziprasidone and m/z 417.0 → 194.012 for the internal standard.

The pharmacokinetic parameters were calculated by standard non-compartmental analysis of the concentration–time data using Kinetica® Version 4.4 (Thermo Fisher Scientific, Woburn, MA).

3. Results

3.1. In vitro characterization of ziprasidone–SBECD complex

3.1.1. Optical microscopy under polarized light

No birefringence was seen with formulation A or SBECD alone, indicating an absence of crystallinity. In contrast, and as expected, birefringence was seen with a physical mixture of ziprasidone mesylate and SBECD. These observations are consistent with formulation A being completely amorphous.

3.1.2. Powder X-ray diffraction (PXRD)

The PXRD patterns for formulation A, a physical mixture of the crystalline drug and SBECD, and the single components (molar ratio of 1:1.3), and each of the components are shown in Fig. 2.

The PXRD patterns of formulation A and SBECD alone indicate that they are both amorphous while the physical mixture of SBECD and ziprasidone mesylate showed diffraction peaks that matched those of the crystalline ziprasidone mesylate salt.

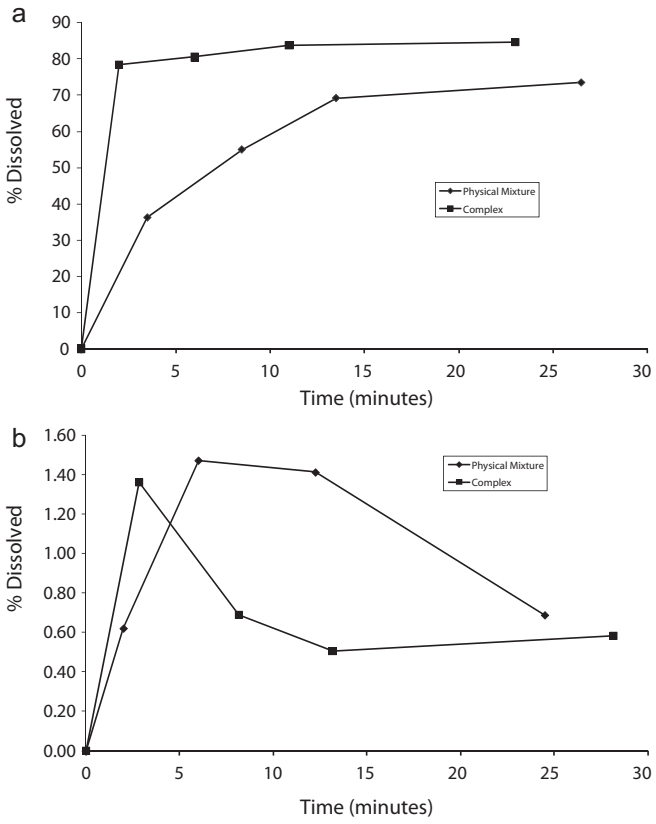

Fig. 4. (a) Dissolution profile of formulation A compared to the physical mixture at pH 4.0. (b) Initial dissolution profile of formulation A compared to the physical mixture at pH 7.4.

3.1.3. Solid state NMR

The proton T1 relaxation times for formulation A, a physical mixture of ziprasidone mesylate with SBECD, lyophilized complexes with lower ratios of ziprasidone to SBECD, SBECD alone, and amorphous drug (prepared by spray drying) are compared in Fig. 3. The broad peaks and the closely correlated relaxation times for formulation A that were distinct from pure controls indicate formation of a molecular complex with an intimate molecular level interaction of ziprasidone with SBECD. This result is consistent with previous solution and solid-state NMR studies (Hong et al., 2011; Kim et al., 1998).

3.1.4. Dissolution study

The dissolution profiles of formulation A and the physical mixture in pH 4.0 and pH 7.4 media are shown in Fig. 4(a and b).

At pH 4.0, formulation A dissolved very rapidly, with almost 80% of the ziprasidone dissolved at the first time point (less than 2 min). In contrast, the physical mixture required about 15 min for 70% of the ziprasidone to dissolve. No precipitate was observed at pH 4.0 after 24 h.

At pH 7.4, formulation A dissolved rapidly, exhibiting a maximum ziprasidone concentration in solution (C_{max}) at the first time point collected. However, the concentration subsequently declined rapidly due to precipitation of ziprasidone. The precipitate was confirmed to be the free base using PXRD and crystal morphology in a separate study. This behavior is expected due to the high energy amorphous formulation supersaturating the media without the presence of precipitation inhibitors at a pH which

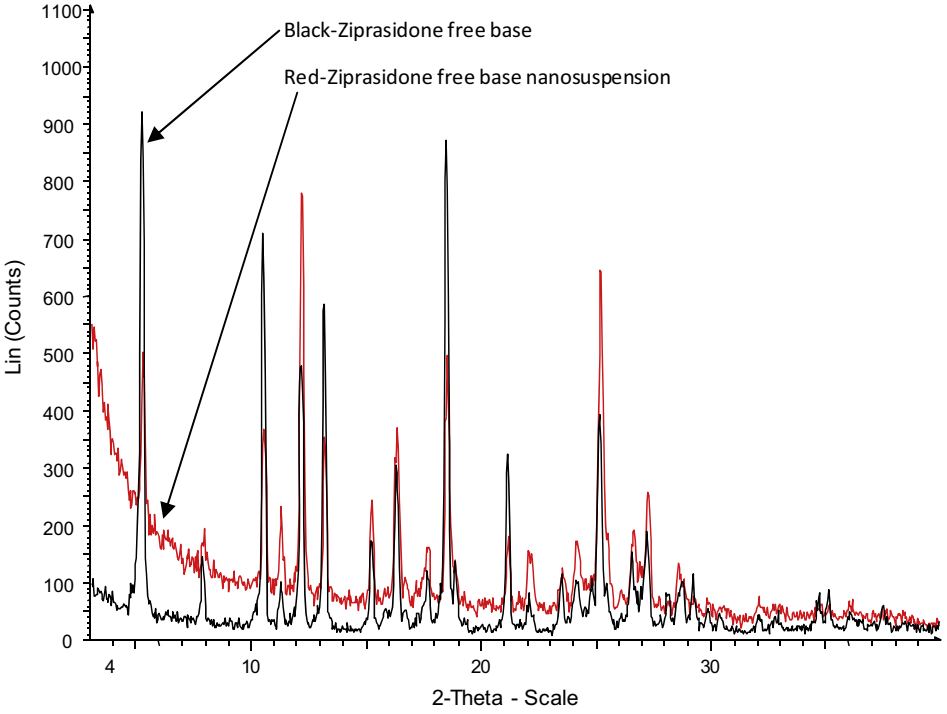

Fig. 5. PXRD pattern of formulation B compared to free base ziprasidone.

predominantly results in precipitation of the low energy form of the drug. In the case of the physical mixture, the slower dissolution of a crystalline drug form resulted in a later C_{max}. The concentrations in the dissolution media from both formulation A and the physical mixture reached the drug's equilibrium solubility (~0.5 μg/mL) at this pH (unpublished results) in less than 30 min.

Thus, at pHs above and below the drug's pK_a, formulation A shows rapid dissolution of ziprasidone. However, the gains in dissolution rate are followed *in vitro* by a rapid transformation to ziprasidone free base at pHs above the pK_a of the drug. Since the permeability of ziprasidone is high once dissolved, the kinetic dissolution enhancement from formulation A was deemed to be sufficient justification for attempting fasted state *in vivo* studies.

3.2. In vitro characterization of formulation B

3.2.1. Particle size and stability

The volume mean diameter, *D*[4,3], of a representative batch of formulation B as a function of storage time at 5 °C is summarized in Table 2. Initially, the *D*[4,3] was 274 nm and the particle size distribution was monomodal with all particles below 752 nm. The physical stability of formulation B was excellent; there was no change in the *D*[4,3] over a period of more than 36 days and the particle size distribution remained monomodal.

Table 2
Mean particle size as a function of storage time for a representative batch of formulation B.

Storage time (days after preparation)	Volume mean diameter *D*[4,3] (nm)
0	274
4	281
7	271
16	268
36	274

3.2.2. Powder X-ray diffraction (PXRD)

The form of ziprasidone in formulation B was confirmed to have remained the crystalline free base form by matching the PXRD pattern with that of the drug substance as shown in Fig. 5.

3.2.3. Dissolution studies

Dissolution testing of formulation B was attempted in a flow-through dissolution system. However, due to the high dose (40–80 mg) and the extremely low aqueous solubility of ziprasidone in neutral pH media, sink conditions were difficult to achieve. Also, using a large dissolution volume increased the analytical challenges in quantitatively measuring drug concentration in the dissolution media. Although not shown by dissolution studies, an enhanced dissolution rate due to the sub-micron small particle size was expected based on surface area enhancement and an increase in saturation solubility might be expected based on the presence of some amorphous drug (Junghanns and Mueller, 2008).

3.3. In vitro characterization of formulation C

3.3.1. Scanning electron microscopy

Scanning electron micrograph images of the ingoing jet-milled ziprasidone HCl drug substance and formulation C are shown in Fig. 6. The particles of formulation C show a spherical and wrinkled appearance, indicating the drug substance is embedded within the film-forming polymer matrix.

3.3.2. Powder X-ray diffraction

Fig. 7 shows the PXRD patterns for a sample of the jet-milled ziprasidone HCl and formulation C. The X-ray diffraction patterns confirmed that the drug form in formulation C was the same crystalline form as the ingoing drug substance.

3.3.3. Particle size and other physical properties

The particle size distribution of a representative lot of the jet-milled ziprasidone HCl and formulation C are shown in Fig. 8. The volume mean diameter of the jet-milled ziprasidone HCl was

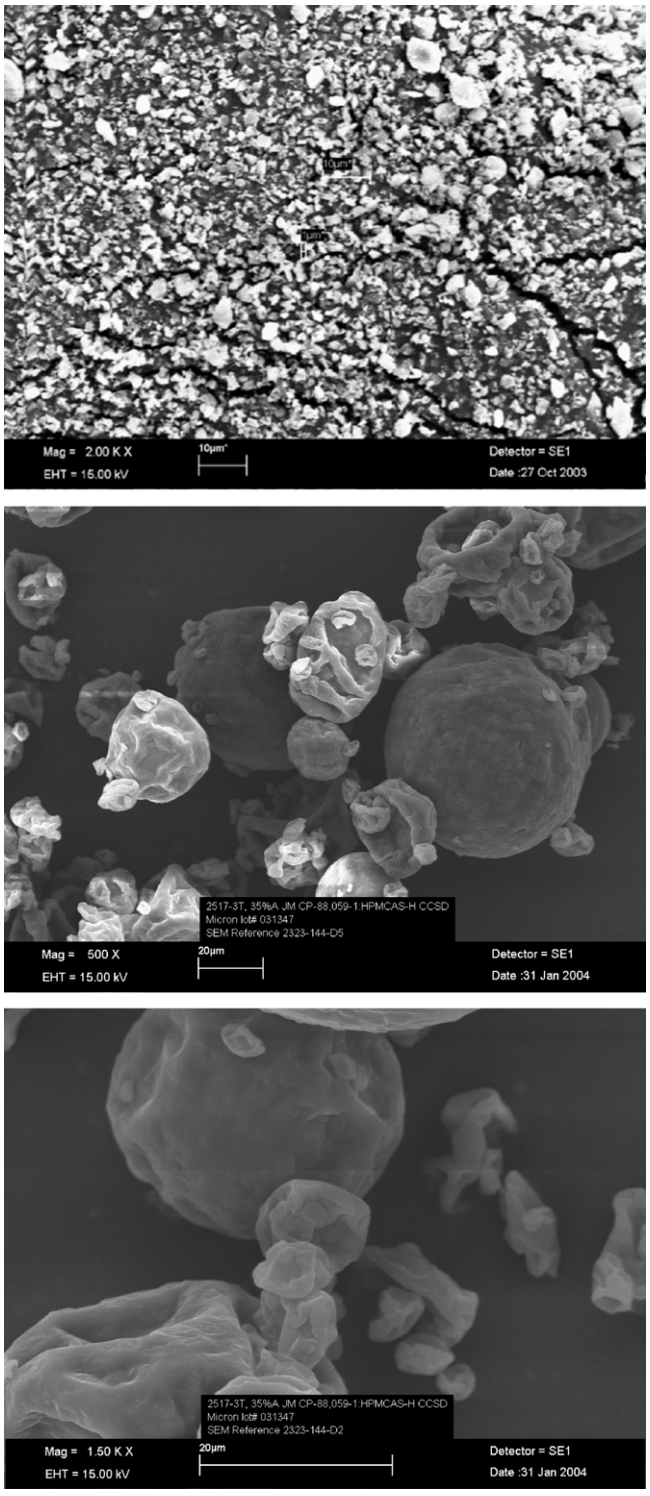

Fig. 6. SEM images of (a) jet-milled ziprasidone HCl API at 2000-fold magnification and formulation C at (b) 500-fold and (c) 1500-fold magnifications.

2.2 μm, and the mean particle size of formulation C was about 44 μm. The bulk and tapped specific volume of formulation C were 4.14 cc/g and 2.65 cc/g, respectively.

3.3.4. Membrane permeation test

The results of the *in vitro* membrane permeation test for formulation C and the jet-milled ziprasidone HCl are shown in Fig. 9. Formulation C showed approximately a 1.5-fold higher flux of free drug across the membrane over the first 60 min of the test

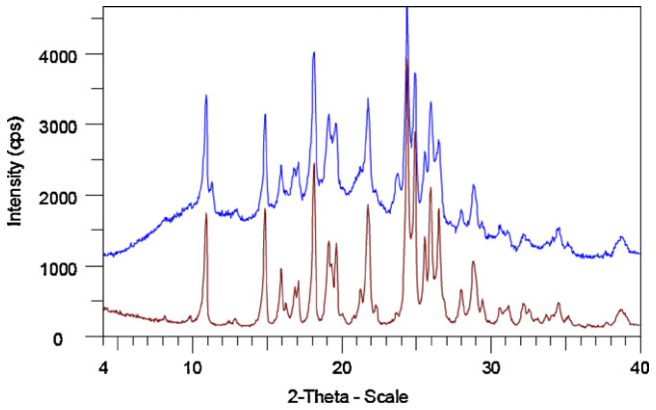

Fig. 7. PXRD diffractograms for formulation C (top) and the ingoing jet-milled crystalline drug (bottom).

compared to the jet-milled ziprasidone HCl and a 2-fold higher total recovery, *i.e.*, percent of drug dosed that permeated the membrane in a 6 h period. These improvements in performance are likely attributed to the precipitation-inhibiting properties of HPMCAS-H resulting in higher free drug concentrations for longer periods of time.

3.4. Pharmacokinetic studies in dogs

The mean ziprasidone concentration *versus* time profiles (normalized to a 40 mg dose) following oral administration of the ziprasidone commercial blend (reference) to fed dogs and the three test formulations (formulations A, B, and C) to fasted dogs are shown in Fig. 10(a) (semi-log plot) and Fig. 10(b) (linear plot). The data for formulation B showed very large inter-individual variability (%CV greater than 100%). Compared to the reference capsule dosed in the fed state, three of the six dogs dosed with formulation B in the fasted state showed exposures that were 11-fold, 8-fold, and 2-fold higher, respectively. This is counter to previous data showing that ziprasidone HCl from the commercial capsule is nearly completely absorbed in the fed state. Dosing and analysis errors were ruled out as potential explanations for this high variability. Therefore, we report the data for formulation B by

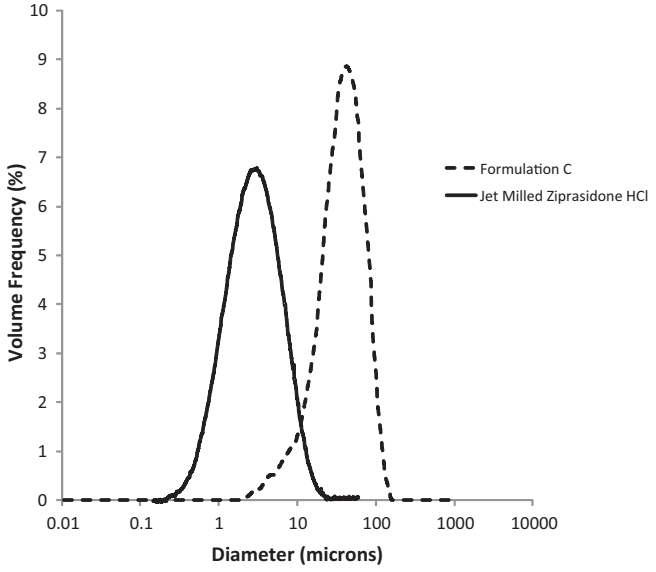

Fig. 8. Particle size distribution of a representative lot of the jet-milled ziprasidone HCl drug substance and formulation C.

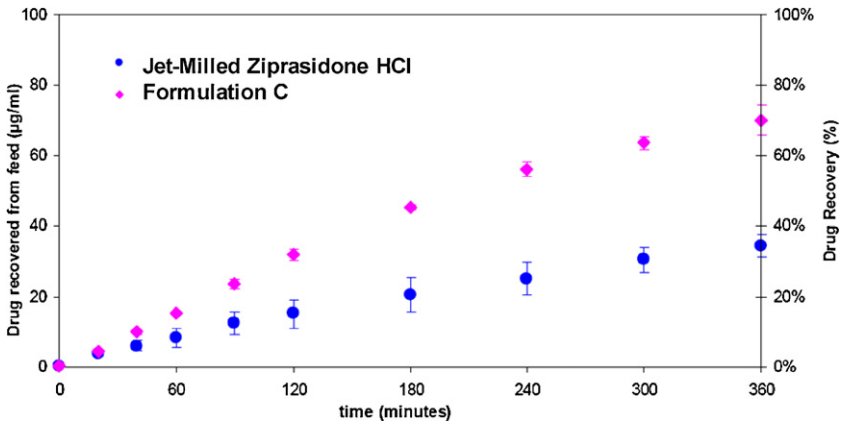

Fig. 9. Membrane permeation profile of formulation C compared with jet-milled crystalline ziprasidone HCl.

Table 3
Summary of serum ziprasidone pharmacokinetic parameter values in beagle dogs.

	T_{max} (h) mean (SD)	C_{max} (ng/mL) geometric mean (%CV)	AUC_{last} ng h/mL	AUC_{inf} ng h/mL
Commercial capsule (fed)	2.3 (1.4)	852 (53)	4677 (46)	4832 (46)
Formulation A	1.2 (0.4)	585 (33)	3158 (18)	3289 (19)
Formulation B[a]	1.2 (0.8)	573 (46)	4646 (39)	5514 (45)
Formulation C	2.3 (1.9)	204 (39)	1125 (26)	1176 (26)

Note: T_{max} values are reported as arithmetic mean (standard deviation). C_{max}, AUC_{last}, and AUC_{inf} values are reported as geometric mean (%CV).

[a] 3 dogs eliminated from the analysis of formulation B. With all six dogs included, the parameters would be: T_{max} = 1.1 h (0.6), C_{max} = 1720 ng/mL (128), AUC_{last} = 11,917 ng h/mL (126), and AUC_{inf} = 13,509 ng h/mL (120).

considering the average of all six dogs and also by omitting the three dogs which showed unusually high exposures.

The corresponding mean dose-normalized pharmacokinetic parameters for all formulations are tabulated in Table 3 and comparisons of the ratios of the pharmacokinetic parameters of the test formulations to the control formulation are presented in Table 4.

Overall, formulation B dosed in the fasted state exhibited the highest oral exposure relative to the reference capsule formulation dosed in the fed state, with AUC_{inf} about 5-fold higher (all six dogs) or about 1.1-fold higher (3 dogs). The geometric mean C_{max} for formulation B was about 4-fold higher (six dog analyses) or slightly lower (67.3%) (3 dog analyses) than the reference capsule formulation dosed in the fed state.

Formulation A also showed improved absorption in the fasted state with AUC_{inf} and C_{max} values about 70% of the values for the reference capsule dosed in the fed state. Formulation C did not show improved absorption in the fasted state, with AUC_{inf} and C_{max} values of about 25% of the reference capsule dosed in the fed state.

The T_{max} values, summarized in Table 3, did not show a significant trend. But, it was interesting to note that the two formulations with enhanced exposure in the fasted state, formulation A and formulation B, exhibited shorter T_{max} values compared to the reference formulation by about 1 h.

Table 4
Summary of comparisons of pharmacokinetic parameter values – test formulations (fasted) *versus* the reference commercial capsule (fed).

	C_{max} ratio	AUC_{last} ratio	AUC_{inf} ratio
Formulation A (fasted) *vs* commercial capsule (fed)	68.4%	67.5%	68.1%
Formulation B[a] (fasted) *vs* commercial capsule (fed)	67.3%	99.3%	114.1%
Formulation C (fasted) *vs* commercial capsule (fed)	23.9%	24.1%	24.3%

[a] 3 dogs eliminated from the analysis of formulation B. With all six dogs included, the ratios would be: C_{max} ratio = 403.8%, AUC_{last} ratio = 509.5%, and AUC_{inf} ratio = 559.1%.

4. Discussion

Because many new drug candidates have poor aqueous solubilities, there have been significant advances in developing solubilization technologies to improve bioavailability. However, there are very few comparative studies, and there is very little guidance available on which technologies may be optimally used to eliminate a food effect. Therefore, in this study, we investigated three approaches to increase ziprasidone absorption in the fasted state in an attempt to reduce or eliminate its food effect.

The ziprasidone mesylate amorphous complex (formulation A) was selected because it showed an improved dissolution rate and high kinetic solubility at intestinal pH. However, it should be recognized that some amorphous formulations have the potential to generate high enough concentrations of supersaturated drug to result in precipitation of a lower solubility drug form such as the free base. Several *in vitro* experimental techniques were used to characterize the complex. Techniques such as optical microscopy and PXRD confirmed the amorphous nature of the lyophilized solid form, but these techniques cannot conclusively prove that it was an inclusion complex rather than an amorphous physical mixture of the drug and SBECD. However, the SS-NMR data confirmed the presence of an inclusion complex and the dissolution data indicated enhanced solubility.

The ziprasidone free base nanosuspension (formulation B) was selected based on an improved dissolution rate due to the high crystal surface area obtained *via* nanosizing along with acceptable physical stability. This formulation would be expected to have a lower potential for supersaturating at intestinal pH, assuming that the drug is not completely dissolved while the drug crystals transit through the stomach. Therefore, this formulation would be expected to yield rapid dissolution without a potential for

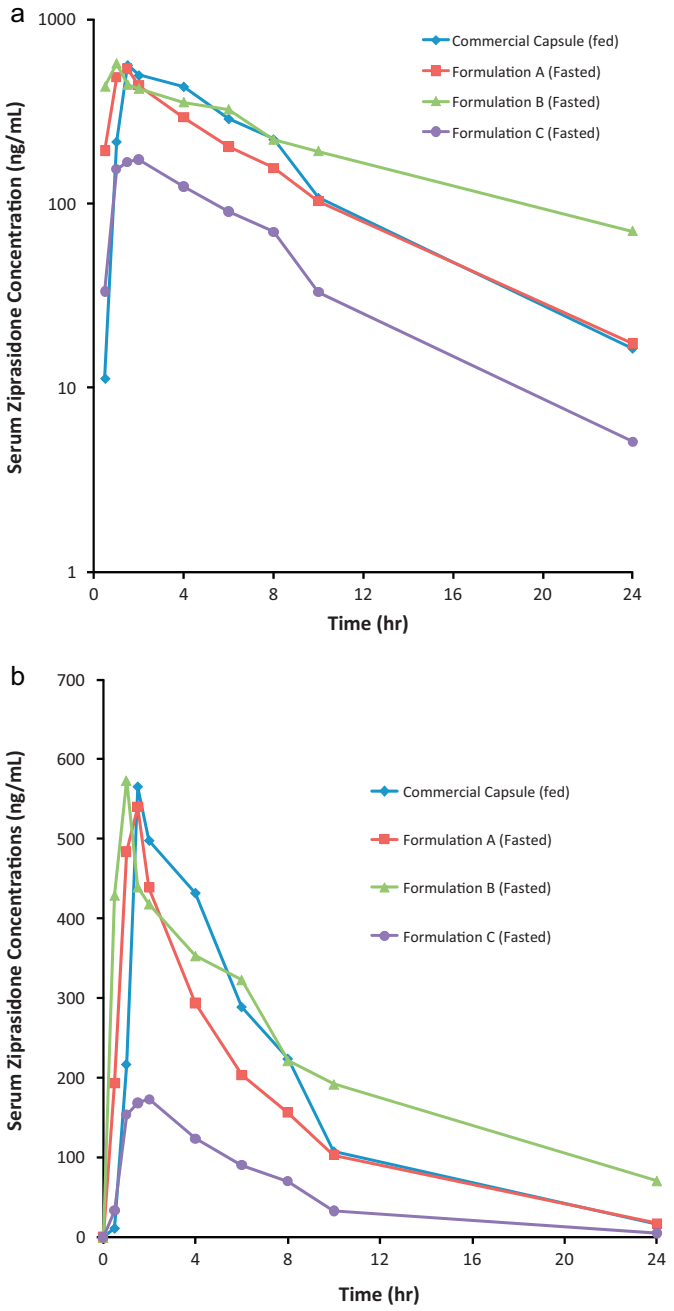

Fig. 10. (a) Mean dose-normalized serum ziprasidone concentration *versus* time profiles (semi-log plot) following administration of the commercial capsule (reference) in the fed state and the test formulations in the fasted state. Note: three dogs omitted from the formulation B data. (b) Mean dose-normalized serum ziprasidone concentration *versus* time profiles (linear plot) following administration of the commercial capsule (reference) in the fed state and the test formulations in the fasted state. Note: three dogs omitted from the formulation B data.

precipitation. An *in vitro* test for dissolution enhancement was not possible because of the challenges in maintaining a sink.

The ziprasidone HCl – CCSD formulation (formulation C) was selected based on a combination of slightly increased dissolution rate resulting from a smaller particle size of the ziprasidone HCl compared to the commercial capsule (*i.e.*, jet-milled drug), a high energy salt form relative to the free base formulation, and inclusion of HPMCAS-HG as a precipitation inhibitor. The *in vitro* characteristics of this formulation were consistent with this rationale; the membrane permeation test showed enhanced flux relative to the bulk HCl salt form alone, indicating the dissolution rate enhancement and precipitation inhibition did yield higher *in vitro* solubility of drug for longer periods of time.

The *in vitro* methods used to characterize the three formulations in this study were selected specifically for each solubilization technology and formulation. Different methods were needed because of the different solubilizing excipients and particle size reduction or amorphous form generation technologies employed. For example, if bile salts were used to simulate dissolution in a fed state media for the cyclodextrin complex, a known interaction between bile salts and cyclodextrin would have confounded the precipitation kinetics and subsequent dissolution results. Similarly, because lecithin was used as a stabilizer for the nanosuspension, the use of lecithin additionally in a fed-state dissolution medium would likely have altered the results.

It would have been ideal if one set of *in vitro* methods could have been used to compare and contrast all three formulations because it would have enabled a more complete understanding of the mechanistic means by which any one of these approaches was able to achieve improved *in vivo* performance. It would have also allowed us to potentially establish a direct correspondence between the *in vitro* and *in vivo* performance. This limitation underscores the current realities of pharmaceutical development in an industrial setting. The availability of a complete and internally consistent data set is usually limited, requiring the application of interpolation and scientific judgment in the practice of pharmaceutical research and development.

The pharmacokinetic studies in dogs showed that the three formulations performed differently. Formulation B yielded the best fasted state absorption enhancement, indicating that improved dissolution rate with a low potential for precipitation might be the best combination of properties to improve ziprasidone absorption in the fasted state. The *in vivo data* for formulation B was highly variable, but the mean summary parameters were comparable to the control capsule dosed in the fed state after three dogs with anomalously high ziprasidone concentrations were eliminated from the analysis.

Formulation A also showed increased absorption of ziprasidone in the fasted state compared to the commercial capsule. Because of the solubilization technology used, ziprasidone likely went quickly into solution and was absorbed, which is consistent with the observed shorter T_{max}. However, the extent of absorption was less than the control capsule dosed in the fed state, indicating that precipitation observed *in vitro* at intestinal pH media may also have occurred under *in vivo* conditions.

Formulation C did not show enhanced absorption in the fasted state compared to the commercial capsule control. This may have been due to one or more of the following: (1) the crystal size was not sufficiently small to enhance the dissolution rate of the HCl drug form, (2) the selection of HPMCAS-H with its dissolution pH of 6.8 yielded a slow drug dissolution rate in the canine intestinal environment, (3) the encapsulation of the milled crystals into larger CCSD spray dried particles resulted in slow dissolution rate, and (4) the drug form did dissolve rapidly as intended but precipitated to the free base form, either in the diffusional boundary layer or in bulk media, yielding the lower-than-expected performance.

In humans, both the ziprasidone–SBECD complex and the ziprasidone nanosuspension showed improved absorption in the fasted state (Thombre et al., 2011) while the CCSD formulation did not (Curatolo et al., 2009). Thus, the *in vivo* performance in dogs was qualitatively similar with the *in vivo* performance of these formulations in humans.

5. Conclusions

Three solubilization technologies were investigated in an attempt to improve fasted state absorption and thereby reduce the

food effect of ziprasidone: formulation A was a lyophilized powder containing ziprasidone mesylate and SBECD, formulation B was a nanosuspension of ziprasidone free base, and formulation C consisted of jet-milled ziprasidone HCl spray dried with HPMCAS, a polymer that inhibits precipitation of dissolved drug in neutral pH media. *In vitro* characterization confirmed that formulation A was an amorphous inclusion complex of ziprasidone in SBECD with enhanced dissolution compared to a physical mixture of the two components. *In vitro* studies also confirmed that formulation B was comprised of ziprasidone free base crystals with a mean particle size of less than about 300 nm and good physical stability. Formulation C showed increased solubilization in a membrane permeation test.

The three formulations were characterized *in vivo* in a pharmacokinetic study in dogs. When compared with ziprasidone HCl commercial capsules dosed in the fed state, both formulation A and formulation B yielded some enhancement in the ziprasidone exposure in the fasted state, indicating that they had the potential to reduce the food effect in humans, resulting in significant patient benefit.

Disclosure

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References

Babu, N.J., Nangia, A., 2011. Solubility advantage of amorphous drugs and pharmaceutical cocrystals. Cryst. Growth Des. 11, 2662–2679.
Bernardo, M., Azanza, J.R., Rubio-Terres, C., Rejas, J., 2006. Cost-effectiveness analysis of schizophrenia relapse prevention: an economic evaluation of the ZEUS (ziprasidone-extended-use-in-schizophrenia) study in Spanish. Clin. Drug Invest. 26, 447–457.
Bernardo, M., Azanza, J.R., Rubio-Terres, C., Rejas, J., 2007. Cost-effectiveness analysis of the prevention of relapse of schizophrenia in the longitudinal study ziprasidone extended use in schizophrenia (ZEUS). Actas Esp. Psiquiatr. 35, 259–262.
Bobes, J., Canas, F., Rejas, J., Mackell, J., 2004. Economic consequences of the adverse reactions related with antipsychotics: an economic model comparing tolerability of ziprasidone, olanzapine, risperidone, and haloperidol in Spain. Prog. Neuropsychopharmacol. Biol. Psychiatry 28, 1287–1297.
Brewster, M.E., Loftsson, T., 2007. Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. 59, 645–666.
Curatolo, W.J., Herbig, S.M., Thombre, A.G., Shah, J.C., Shamblin, S.L., Lukas, T., Caldwell, W.B., Friesen, D.T., Lyon, D.K., Craig, C.D., 2009. Methods, dosage forms, and kits for administering ziprasidone without food. (Pfizer Inc., USA). Application: WO 2009109844 A1.
Daniel, D.G., 2003. Tolerability of ziprasidone: an expanding perspective. J. Clin. Psychiatry 64, 40–49.
Fleisher, D., Bong, R., Stewart, B.H., 1996. Improved oral drug delivery: solubility limitations overcome by the use of prodrugs. Adv. Drug Deliv. Rev. 19, 115–130.
Friesen, D.T., Shanker, R., Crew, M., Smithey, D.T., Curatolo, W.J., Nightingale, J.A.S., 2008. Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview. Mol. Pharm. 5, 1003–1019.
Gandelman, K., Alderman, J.A., Glue, P., Lombardo, I., La Badie, R.R., Versavel, M., Preskorn, S.H., 2009. The impact of calories and fat content of meals on oral ziprasidone absorption: a randomized, open-label, crossover trial. J. Clin. Psychiatry (Memphis, TN, United States) 70, 58–62.
Geodon® [U.S. Prescribing Information including Patient Summary of Information], Pfizer Inc., Revised 2009.
Greenberg, W.M., Citrome, L., 2007. Ziprasidone for schizophrenia and bipolar disorder: a review of the Clinical trials. CNS Drug Rev. 13, 137–177.
Hamelin, B.A., Allard, S., Laplante, L., Miceli, J., Wilner, K.D., Tremblay, J., Lebel, M., 1998. The effect of timing of a standard meal on the pharmacokinetics and pharmacodynamics of the novel atypical antipsychotic agent ziprasidone. Pharmacotherapy 18, 9–15.
Harrison, T.S., Scott, L.J., 2006. Ziprasidone: a review of its use in schizophrenia and schizoaffective disorder. CNS Drugs 20, 1027–1052.
Hauss, D.J., 2007. Oral lipid-based formulations. Adv. Drug Deliv. Rev. 59, 667–676.
Hong, J., Shah, J.C., McGonagle, M.D., 2011. Effect of cyclodextrin derivation and amorphous state of complex on accelerated degradation of ziprasidone. J. Pharm. Sci. 100, 2703–2716.
Humberstone, A.J., Charman, W.N., 1997. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv. Drug Deliv. Rev. 25, 103–128.
Janiszewski, J.S., Fouda, H.G., Cole, R.O., 1995. Development and validation of a high-sensitivity assay for an antipsychotic agent, CP-88,059, with solid-phase extraction and narrow-bore high-performance liquid chromatography. J. Chromatogr. B: Biomed. Appl. 668, 133–139.
Junghanns, J.-U.A.H., Mueller, R.H., 2008. Nanocrystal technology, drug delivery and clinical applications. Int. J. Nanomed. 3, 295–309.
Kesisoglou, F., Panmai, S., Wu, Y., 2007. Nanosizing – oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.
Kim, Y., Oksanen, D.A., Massefski Jr., W., Blake, J.F., Duffy, E.M., Chrunyk, B., 1998. Inclusion complexation of ziprasidone mesylate with beta-cyclodextrin sulfobutyl ether. J. Pharm. Sci. 87, 1560–1567.
Lincoln, J., Stewart Mark, E., Preskorn Sheldon, H., 2010. How sequential studies inform drug development: evaluating the effect of food intake on optimal bioavailability of ziprasidone. J. Psychiatr. Pract. 16, 103–114.
Melander, A., 1978. Influence of food on the bioavailability of drugs. Clin. Pharmacokinet. 3, 337–351.
Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120.
Miceli, J.J., Wilner, K.D., Hansen, R.A., Johnson, A.C., Apseloff, G., Gerber, N., 2000. Single- and multiple-dose pharmacokinetics of ziprasidone under nonfasting conditions in healthy male volunteers. Br. J. Clin. Pharmacol. 49, 5S–13S.
Patel, N.C., Keck Jr., P.E., 2006. Ziprasidone: efficacy and safety in patients with bipolar disorder. Expert Rev. Neurotherapeutics 6, 1129–1138.
Perkins, D.O., 2002. Predictors of noncompliance in patients with schizophrenia. J. Clin. Psychiatry 63, 1121–1128.
Porter, C.J.H., Pouton, C.W., Cuine, J.F., Charman, W.N., 2008. Enhancing intestinal drug solubilization using lipid-based delivery systems. Adv. Drug Deliv. Rev. 60, 673–691.
Schmidt, L.E., Dalhoff, K., 2002. Food–drug interactions. Drugs 62, 1481–1502.
Serajuddin, A.T.M., 2007. Salt formation to improve drug solubility. Adv. Drug Deliv. Rev. 59, 603–616.
Shah, J.C., Shah, P.S., Wisniecki, P., Wagner, D.R., 2008. Injectable depot formulations and methods for providing sustained release of nanoparticle compositions. (Pfizer Inc., USA). Application: US 20080305161 A1.
Singh, B.N., 1999. Effects of food on clinical pharmacokinetics. Clin. Pharmacokinet. 37, 213–255.
Singh, B.N., Malhotra, B.K., 2004. Effects of food on the clinical pharmacokinetics of anticancer agents: underlying mechanisms and implications for oral chemotherapy. Clin. Pharmacokinet. 43, 1127–1156.
Stahl, S.M., Shayegan, D.K., 2003. The psychopharmacology of ziprasidone: receptor-binding properties and real-world psychiatric practice. J. Clin. Psychiatry 64, 6–12.
Stella, V.J., Nti-Addae, K.W., 2007. Prodrug strategies to overcome poor water solubility. Adv. Drug Deliv. Rev. 59, 677–694.
Tang, J.-l., Sun, J., He, Z.-G., 2007. Self-emulsifying drug delivery systems: strategy for improving oral delivery of poorly soluble drugs. Curr. Drug Ther. 2, 85–93.
Thombre, A.G., Herbig, S.M., Alderman, J.A., 2011. Improved ziprasidone formulations with enhanced bioavailability in the fasted state and a reduced food effect. Pharm. Res. 28, 3159–3170.
Toothaker, R.D., Welling, P.G., 1980. The effect of food on drug bioavailability. Annu. Rev. Pharmacool. Toxicol. 20, 173–199.
Usha, Y.N., Angel, T.T., Udupa, N., 2010. Nanotechnology: perspectives on solubility/bioavailability enhancement. Pharma Rev. 8, 59–66.
Welling, P.G., 1996. Effects of food on drug absorption. Annu. Rev. Nutr. 16, 383–415.
Zimbroff Dan, L., Allen Michael, H., Battaglia, J., Citrome, L., Fishkind, A., Francis, A., Herr Daniel, L., Hughes, D., Martel, M., Preval, H., Ross, R., 2005. Best clinical practice with ziprasidone IM: update after 2 years of experience. CNS Spectr. 10, 1–15.