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RESEARCH PAPER

Effect of Physicochemical and Formulation Variables on the In Vivo Absorption of ABT-761

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ABSTRACT

ABT-761 is a 5-lipoxygenase inhibitor developed for the treatment of asthma. The present study was undertaken to evaluate different crystal forms of ABT-761 and their impact on in vitro and in vivo performance in capsule formulations. Two crystal forms of ABT-761, hemihydrate and non-solvate from different sources, were characterized by thermal analysis, x-ray powder diffraction, moisture sorption, and intrinsic dissolution studies. An in vitro test was performed to assess the effect of formulation and drug from different sources on drug release. Crossover design was also used to evaluate oral bioavailability of ABT-761 hemihydrate formulations in beagle dogs. Plasma concentrations of ABT-761 were analyzed using a reverse-phase high-performance liquid chromatography (HPLC) assay. It was found that in vivo oral absorption as well as in vitro dissolution of ABT-761 were influenced by different formulations. Capsule formulations of ABT-761 hemihydrate are bioequivalent to the solution formulation in terms of extent of absorption, but formulation and the method of granulation preparation can have a major impact on the absorption rate. In conclusion, a single crystal form of ABT-761, i.e., hemihydrate, is preferred for subsequent product development. However,

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exposure of the drug to conditions that may facilitate phase transformation should be avoided.

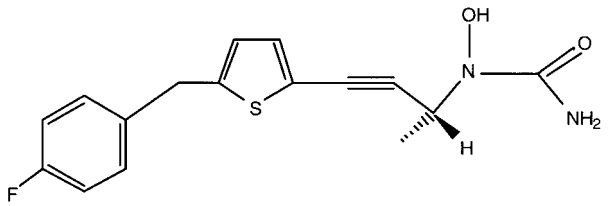
Key Words: *Beagle dog; Bioavailability; Crystal form; Formulation; 5-Lipoxygenase inhibitor*

INTRODUCTION

ABT-761, R(+) *N*-[3-[5-(4-fluorophenoxy)-thiophen-2-yl]-1-methyl-2-propynyl]-*N*-hydroxyurea (Fig. 1), a potent new 5-lipoxygenase inhibitor, was developed for alleviating airway constriction in asthma. 5-Lipoxygenase is an enzyme that converts arachidonic acid to 5-hydroperoxy-eicosa-6,8,11,14-tetraenoic acid (5-HPETE) in the pathway leading to the production of leukotrienes (1). Inhibition of leukotriene synthesis can have many potential therapeutic benefits for conditions in which leukotriene synthesis is elevated, such as asthma (2).

ABT-761 is crystalline and practically insoluble in aqueous medium (3.6 μg/mL). However, its gastrointestinal permeability is expected to be high due to its favorable lipophilicity ($\log P = 3.5$) and small molecular weight. According to the biopharmaceutical drug classification scheme, ABT-761 is a Case II drug, i.e., low solubility/high permeability (3). Drugs in this class often exhibit variable absorption due to various physicochemical, formulation, and in vivo variables that can affect the dissolution performance (3).

ABT-761 exists in two different isolable crystal forms differentiated by their degrees of hydration: hemihydrate and non-solvate forms (4). The existence of different solid-state forms of a compound implies different interaction energies in the solid state with a likelihood of different physicochemical properties that may have potential effects on formulations, processing, and bioavailability. The present studies were undertaken to investigate physicochemical characteristics of ABT-761 from different sources and to evaluate oral bioavailability of different capsule formulations of the hemihydrate relative to a solution.



$C_{16}H_{15}FN_2O_2S$; MW = 318.37

Figure 1. Chemical structure of ABT-761.

EXPERIMENTAL

Materials and Equipment

The following materials and equipment were used in the study: ABT-761 Form I, hemihydrate (Source I, Chemical and Agriculture Division, Abbott Laboratories); ABT-761 Form II, non-solvate (Source II, Pharmaceutical Product Division, Abbott Laboratories); ABT-761 mixed form, a mixture of hemihydrate and non-solvate (Source III, Finorga, France). A-86531 (internal standard, Abbott Laboratories). All other chemicals and reagents were either AR or HPLC grades and used as received. A Mettler DSC 30 differential scanning calorimeter, Mettler TG50 thermogravimetric analyzer, Scintag XDS 2000 x-ray powder diffraction system equipped with a 2 kW normal focus copper x-ray tube and a liquid nitrogen-cooled germanium solid-state detector, VTI MB-300 G moisture sorption apparatus, and Retsch ball mill were utilized in physicochemical characterization studies. Analysis of samples by high-performance liquid chromatography (HPLC) was performed using a Spectra Physics HPLC pump (KK6374), a Spectra Physics autosampler (IM5517), a Kratos Analytical Spectroflow 783 UV detector (IM1195), and a Spectra Physics integrator (IM5518).

Physicochemical Characterization

The following studies were carried out in order to characterize the hemihydrate and non-solvate forms of ABT-761 from different sources.

Thermal Analysis

Differential scanning calorimetry (DSC) experiments were performed with 1–10 mg samples sealed in standard aluminum pans with a single hole

punched in the lid. An empty pan of the same type was used as a reference. The heating rate was 10°C/min. The samples were purged with dry nitrogen (40–50 mL/min). Thermogravimetry (TGA) was performed with a sample configuration, scanning rate, and dry nitrogen flow similar to that used for DSC.

X-ray Powder Diffraction

Samples were scanned continuously from 2° to 40° 2θ at 1°/min. Samples were run in a bulk configuration (1 inch diameter holder) and were spinning during the acquisition of the data. Data were collected on a DEC 3100 MicroVAX computer running Scintag's Diffraction Management System Software (DMS).

Moisture Sorption

Water vapor sorption experiments were performed by initially drying the material of interest (~10 mg) under vacuum at 50°C (±0.5°C) until no further weight change was measurable (< 5 μg for three successive 5-min periods). This condition was held for an additional 30 min. The sample was then exposed to a stepwise increase in relative humidity (RH) from 0 to 95% at 25°C (5% RH steps). Each successive step was initiated when the change in weight at that relative humidity was smaller than 5 μg for three successive 5-min periods. The sample was then taken through the reverse of the stepwise % RH increase.

Milling

ABT-761 was milled using a Retsch ball mill. Samples were placed in both left- and right-hand milling chambers consisting of 10 mL stainless steel mortars and 12 mm diameter stainless steel balls. Milling was carried out for 5 min at a setting of 80.

Karl Fischer Analysis

Analysis for water content was performed on a Mettler DL 35 Karl Fischer titrator utilizing Hydranal Titrant 2 (Riedel de Haen). This study was performed in triplicate after standardization with sodium tartrate 2-hydrate (Hydranal Standard).

Intrinsic Dissolution Testing

Non-disintegrating tablets of either hemihydrate (Source I) or mixed form (Source III) were prepared by direct compression from 75 mg of the crystals with a Carver press under a pressure of 2000 psi for 45 sec using a punch and die set. The intrinsic dissolution was determined under the following conditions: medium = distilled water; volume = 400 mL; temperature = 37±1°C; stirring speed = 60 rpm; sampling interval = 0.17, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hr. ABT-761 in the sample was assayed using a reverse-phase HPLC methodology.

Formulations

Capsule formulations containing GMP lots of ABT-761 from Source I used in bioavailability studies are given in Table 1.

In assessing the effect of formulation and processing on in vivo absorption, two capsule formulations were designed (A and B of Table 1), both containing hemihydrate. Capsule formulation A was prepared by a dry granulation process. The drug,

Table 1

Primary Composition of Capsule Formulations Used to Assess Bioavailability of ABT-761

Component (%)	Formulation A	Formulation B
ABT-761 hemihydrate	10.0	10.0
Water-soluble filler	82.75	79.0
Croscarmellose	2.0	4.0
Povidone K30	0.0	2.0
Talc	4.0	4.0
Magnesium stearate	1.25	1.0
Granulation method	Dry	wet

fillers, and disintegrant were mixed followed by slugging using roller compaction. The compacts were milled using a Fitzmill with knife forward. The milled particles were then blended with the lubricants and filled into hard gelatin capsules. Formulation B was made via wet granulation. ABT-761, fillers, and disintegrant were dry mixed in a high-shear mixer (Collette Gral 10) followed by granulation with a 2% PVP solution. The wet granules were tray-dried overnight, sized, and blended with the lubricants before encapsulation in hard gelatin capsules.

In Vitro Dissolution Test

The in vitro dissolution tests were performed using USP apparatus II (paddle). Nine hundred milliliters of 50 mM SDS solution was used as the dissolution medium at 37±0.5°C. The paddle rotation speed was kept at 50 rpm. Dissolution samples were withdrawn at predetermined time intervals, and replaced with an equal volume of the fresh medium to maintain the total volume constant. Samples were filtered through a filter (0.45 μm) and assayed by a reverse-phase HPLC method. Because of the low solubility of ABT-761, 50 mM solution of SDS was used as the dissolution medium in order to maintain sink conditions during release testing.

In Vivo Studies

Nine beagle dogs weighing 7.6–14.1 kg were used in studying formulations A and B to test the effect of dry vs. wet granulation. The reference formulation was an oral solution containing 0.5 mg/mL of ABT-761 in a water : ethanol : propylene glycol (4 : 3 : 3) solvent system. All dogs were fasted overnight and then fed with one can of dog food (Kalkan Pedigree) 0.5 hr before dosing. A three-way crossover design with 1-week dosing intervals was used for the two capsule formulations and the solution formulation. Drug was orally administered followed by 25 mL of water, then water was provided ad libitum throughout the study. Food was returned after the 8-hr blood sample was taken. Serial blood samples were collected up to 32 hr after single dosing. Plasma samples were immediately separated and frozen at −20°C until assayed.

HPLC Assays

Plasma samples were assayed by the following methods.

(1) Liquid–liquid extraction: 0.5 mL of blood sample were combined with 0.1 mL of the internal standard (A-86531) solution and then mixed with 6 mL of a solution containing methylene chloride : ethanol (9 : 1 v/v). The resulting mixture was shaken at low speed for approximately 20 min. Following centrifugation at 2500 rpm for 10 min, the aqueous layer was aspirated to waste. The organic phase was transferred to a test tube and subsequently evaporated to dryness with a gentle stream of dry air over low heat (< 45°C). The samples were reconstituted with 0.30 mL of methanol : water (3 : 7 v/v) for HPLC analysis.

(2) HPLC method: A reverse-phase HPLC assay was used to determine the concentration of ABT-761 in the plasma samples. A Regis Little Champ C-18 column (50 × 4.6, Spherisorb, 3 μm) was used for the assay. The mobile phase used was tetrahydrofuran : aqueous solution (25 : 75 v/v) at a flow rate of 1.0 mL/min. The aqueous solution contained 0.13% tetramethylammonium perchlorate and 0.075% trifluoroacetic acid. The ultraviolet (UV) detection wavelength was 260 nm.

For each set of blood samples, a calibration curve was constructed with spiked standards and used for calculation of the sample concentrations. Selectivity was assessed by examining peak interference from endogenous matrix components. The limit of quantitation (LOQ) of the assay was determined to be 0.05 μg/mL.

Data Analysis

The area under the plasma concentration–time curve (AUC) from time zero to the last sampling time point t (AUC_t) was calculated by the trapezoidal rule. AUCs from time zero to time infinity were obtained by extrapolation using terminal phase elimination constants. The peak drug concentration (C_{max}) and the time to peak drug concentration (t_{max}) were obtained directly from the data without interpolation. The drug plasma concentration data were used to estimate the in vivo mean residence time (MRT) of the drug following administration of different formulations according to the

following equation (5):

$$\mathrm{MRT} = \frac{\int_0^\infty tC(t)\mathrm{d}t}{\int_0^\infty C(t)\mathrm{d}t} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \quad (1)$$

where AUC and AUMC are the area under the plasma concentration [$C(t)$] curve and the area under the first moment curve, respectively. Both AUC and AUMC were calculated by the trapezoidal rule. Integration from the last time point (t_n) to infinity was estimated by $\mathrm{AUMC} = t_n C(t_n)/k + C(t_n)/k^2$ where k is the terminal rate constant. MRT represents the mean time that drug molecules spend in the body system after oral administration. Analysis of variance (ANOVA) was performed using JMP2.0.5 (SAS Institute, Inc., Cary, NC). The sources of variation included in the model were formulation, subjects, sequence, and period. The two one-sided hypotheses at significance level (α) of 0.05 were tested for AUC_t and $C_{\max}$ by constructing the 90% confidence interval for the ratio between the test and reference averages.

RESULTS AND DISCUSSION

Comparison of the Two Crystal Forms

The events observed in the DSC thermogram of the hemihydrate form of ABT-761 included a desolvation endotherm, followed by two small endotherms at 120 and 130°C, respectively, and an endotherm/exotherm sequence which coincides with the decomposition of the compound. The TGA scan demonstrates a loss of weight of ~3% corresponding to the first DSC endotherm. This desolvation of the hemihydrate (Form I) results in the formation of an intermediate phase. The first small endotherm observed corresponds to the conversion to a non-solvate form of ABT-761 (Form II). The next event (second small endotherm) results in the formation of an isotropic phase which is then shown to decompose at the above-mentioned endotherm/exotherm sequence. As shown by DSC cycling experiments, the events up until the first small endotherm are reversible. Once the first small endotherm was reached, a distinct non-solvated phase was created which did not revert to the original hemihydrate upon cooling. A DSC thermogram of non-solvate ABT-761 demonstrated only the endotherm/exotherm sequence associated with decomposition.

X-ray powder diffraction patterns were obtained for both Form I and Form II of ABT-761. Distinct patterns indicative of separate phases were obtained (Fig. 2).

Water Content of the Hemihydrate

The water content of Form I of ABT-761 was first examined by Karl Fischer analysis. The results

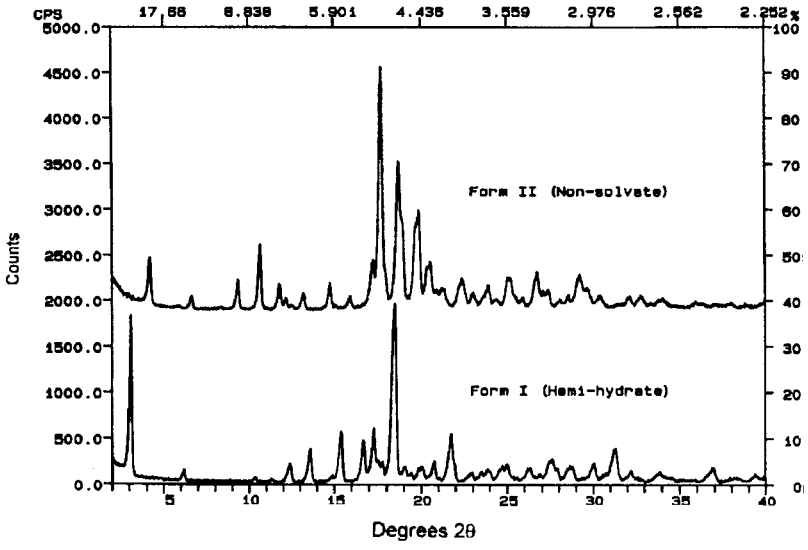


Figure 2. X-ray powder diffraction patterns of hemihydrate and non-solvate ABT-761.

of a triplicate analysis yielded an average of 3.1% (s.d. = 0.62%) water, which is consistent with the theoretical water content of 2.75%.

An additional experiment was performed with the hemihydrate (Form I) using a VTI moisture sorption apparatus. The dried material was found to sorb 2.8% moisture between 10 and 15% RH. An additional 1% moisture was accumulated over the remainder of the experiment. This additional moisture is believed to be non-specifically associated with the solid. As with the Karl Fischer determination, this 2.8% sorption at low RH is consistent with hemihydrate formation.

In a similar experiment, the non-solvated form (Form II) was found to sorb only 1% moisture when exposed to up to 95% RH. None of this moisture was acquired in a stepwise fashion as, with the dried hemihydrate and much like the moisture sorbed with Form I beyond the 0.5 mol of water of hydration, it is believed to be non-specifically associated with this material.

Effect of Milling

The inter-conversion of different crystal forms induced during processing and manufacturing can have a potential impact on the dissolution and bioavailability of a drug. In the present study, conversion from Form I to Form II was found to take place readily when Form I was milled followed by heating. Following milling of the hemihydrate in a ball mill for 5 min, thermal analysis by DSC showed qualitative differences between the original and the milled material. The most noteworthy feature was that the small endotherms originally present were no longer observed. Furthermore, when a DSC cycling experiment was conducted where the milled material was heated to 100°C, upon cooling and reheating, the thermogram was shown to resemble the original thermogram of Form II.

Intrinsic Dissolution Rates

Dissolution from non-disintegrating tablets under fixed hydrodynamic conditions allows one to differentiate intrinsic dissolution properties of particular crystal states from particle size, wetting, and other formulation-related effects. Therefore, the dissolution rate measured from a disk with constant surface area can be considered as the intrinsic dissolution of the crystalline drug. For a dissolving disk under sink conditions with a constant surface area, the dissolution process can be described by (6):

$$W = kC_s t \tag{2}$$

where W is the amount dissolved, C_s is the solubility of the compound at time t, and the constant k includes the surface area of the dissolving disk, the diffusion coefficient, and the diffusion layer thickness. Thus, a plot of amount dissolved as a function of time should be linear for the initial dissolution, and the intrinsic dissolution rate can be calculated from the slope. The intrinsic dissolution rates are 27.6 mg/cm^2/hr for hemihydrate ($R^2 = 0.9958$) and 24.0 mg/cm^2/hr for the mixed form ($R^2 = 0.9997$). The dissolution profiles of the two different lots are shown in Fig. 3. Two-way ANOVA indicates that the dissolution rate of the hemihydrate form is significantly greater than that of the mixed form ($p = 0.011$).

In Vitro Dissolution

Dissolution profiles of capsule formulations A and B are shown in Fig. 4. The initial release rate of formulation B prepared by wet granulation is significantly higher than that of formulation A made from dry granulation. The difference could be due to the minor difference in formulations, but is more likely attributed to different processing conditions. Thus, the milling process used with formulation A may have had an impact on the physical form of the hemihydrate, such as partial conversion from hemihydrate to non-solvate induced by milling. A higher percentage of disintegrant was used in formulation B because it was wet granulated with a binder.

It should be pointed out that incomplete drug release was observed for both formulations at 60 min. However, the labeled potency was recovered for both formulations by continued mixing at an increased agitation speed of 150 rpm for an additional 30 min. The initial burst in the first 15 min followed by slow dissolution in Fig. 4 may be a result of rapid dissolution of fine drug particles and subsequent dissolution of larger particles that may be limited by the diffusion layer for insoluble compounds.

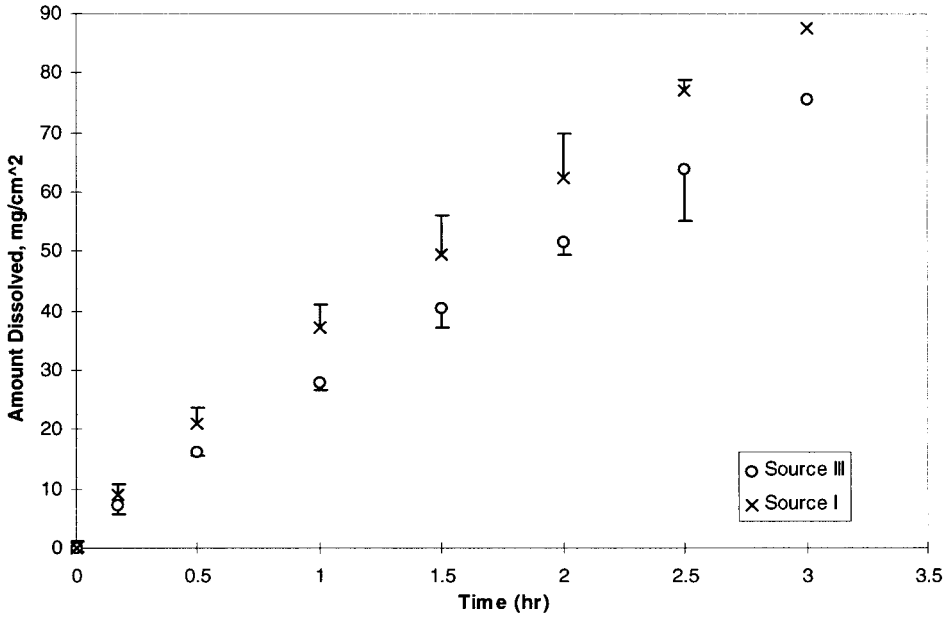


Figure 3. Intrinsic dissolution profiles of hemihydrate from Source I and the mixed form from Source III (mean±SD).

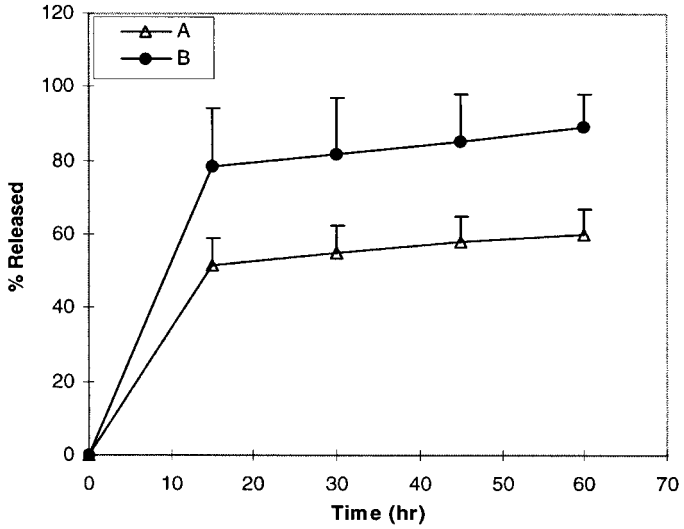


Figure 4. In vitro dissolution of ABT-761 from capsule formulations A and B (mean±SD).

In Vivo Absorption

In order to evaluate the in vivo performance of prototype formulations, in vivo bioavailability of selected formulations was studied in beagle dogs. The dog has been reported to be a reasonable animal model for studying absorption of immediate release oral dosage forms due to its similarities to humans in gastrointestinal physiology (7) and its ability to ingest dosage units developed for human use. However, correlation in oral absorption between dog and human is often found to depend on the physicochemical and biopharmaceutical properties of a particular molecule (8). Therefore, caution should be

exercised if such data is to be extrapolated to humans. Figure 5 shows the mean plasma concentration–time curves following single oral administration of formulations to the dogs. Estimates of the ratios of formulation AUC, C_{max}, and the corresponding 90% confidence intervals are given in Table 2.

Model-independent pharmacokinetic parameters were also calculated and are summarized in Table 2. Elimination rate constants were estimated from the terminal phase of the plasma concentration–time profiles for individual dogs. The mean elimination half-life was found to be within the range 11.28–12.47 hr. The values of total clearance (Cl) of ABT-761 obtained based on Eq. (3) were very close to each other among different formulations:

$$\mathrm{Cl} = \frac{FD}{\int_0^{\infty} C(t)\mathrm{d}t} \tag{3}$$

D is the dose, F is the fraction absorbed.

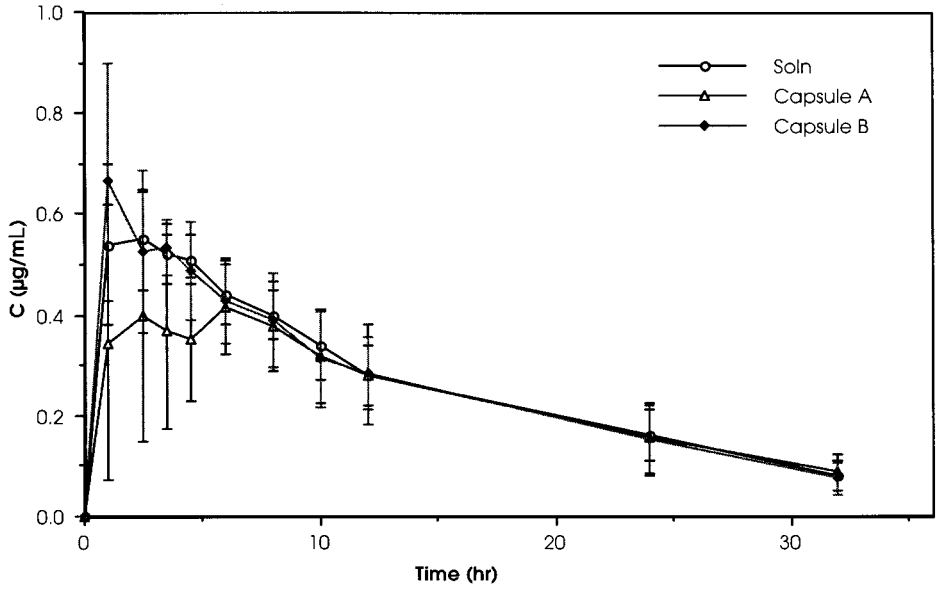


Figure 5. Mean plasma concentration–time profiles of ABT-761 after a single oral dose of capsule and solution formulations to beagle dogs (mean±SD).

Table 2

Average Bioavailability and Pharmacokinetic Parameters of ABT-761 in Beagle Dogs (n = 9)

Parameter	Solution	Capsule A	Capsule B
AUC_{32} (hr µg/mL)	9.62(±2.29)	8.57(±2.14)	9.40(±1.99)
F	1.00(±0.00)	0.89(±0.07)	0.99(±0.15)
90% CI[a]	—	[0.84–0.94]	[0.88–1.11]
C_{max} (µg/mL)	0.62(±0.12)	0.55(±0.06)	0.73(±0.05)
F (C_{max})	1.00(±0.00)	93(±0.21)	1.22(±0.24)
90% CI[a]	—	[0.77–1.09]	[1.04–1.40]
T_{max} (hr)	2.78(±1.09)	3.33(±2.09)	1.39(±1.17)
MRT (hr)	16.16(±3.21)	18.28(±5.07)	15.71(±4.28)
k (hr^{-1})	0.061(±0.012)	0.059(±0.011)	0.068(±0.023)
$t_{1/2}$ (hr)	11.63(±2.10)	12.47(±2.57)	11.28(±3.69)
Cl/F (L/hr kg)	0.10(±0.02)	0.11(±0.03)	0.11(±0.03)

[a]90% Confidence intervals for the ratio of the test and reference formulation.

In the study the effects of formulation and granulation process (dry vs. wet) on oral absorption were evaluated. The estimates of bioavailability of formulations A and B relative to solution were 0.89(±0.07) and 0.99(±0.15), respectively. Based on ANOVA, formulations (p=0.017), period (p=0.043), and subject ($p < 0.001$) were found to be the main sources of variation. The effect of sequence was insignificant (p=0.571). The confidence intervals for the test/reference ratios of the mean AUC were within the equivalent range of bioequivalence. However, it is apparent that absorption of formulation A was extended for approximately 6 hr following dosing, while in vivo absorption of formulation B was rapid. The upper limit of the confidence interval of C_{max} was above 1.25 for capsule B. The lower limit of the confidence interval of C_{max} was below 0.80 for capsule A. Hence, formulations A and B are not bioequivalent to the solution in terms of C_{max}. This may suggest a slightly higher rate of drug absorption from capsule B and a significantly slower absorption from capsule A, which agrees with the rank order observed in in vitro dissolution results. The same trend between formulations A and B was also indicated by the difference in C_{max}/AUC (0.064 vs. 0.078), T_{max} (3.33 vs. 1.39), and MRT (18.28 vs. 15.71) when intrinsic residence times of the molecule are similar as reflected by similar Cl/F.

In general, Case II compounds are more likely subject to significant food effects because of food-induced changes in the GI tract (e.g., mixing, bile flow, and splanchnic blood flow). Thus, minor differences in absorption between formulations often disappear upon food intake. The dosing regimen used in the present study was based on earlier studies that indicated favorable absorption of ABT-761 under non-fasting conditions. Nevertheless, the rates of absorption remain significantly different between formulations A and B, despite the equivalent AUC values in solution. In principle, either formulation may be pursued further based on the extent of absorption. However, formulation A might result in a higher variability because of the extended absorption and the potential impact of its processing on the physical form of ABT-761.

CONCLUSIONS

Dissolution of ABT-761 is influenced by different crystal forms, formulations, and processing conditions. Hence, a single crystal form of ABT-761, i.e., hemihydrate from Source I, is preferred for subsequent product development. Capsule formulations of ABT-761 hemihydrate are bioequivalent to the solution formulation in terms of extent of absorption, but the rate of absorption may depend on the formulation and processing variables. Exposure of the drug to conditions that may facilitate phase transformation, such as milling, should be avoided.

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