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Cryogenic liquids, nanoparticles, and microencapsulation

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Abstract

The biopharmaceutical classification system (BCS) is used to group pharmaceutical actives depending upon the solubility and permeability characteristics of the drug. BCS class II compounds are poorly soluble but highly permeable, exhibiting bioavailability that is limited by dissolution. The dissolution rate of BCS class II drug substances may be accelerated by enhancing the wetting of the bulk powder and by reducing the primary particle size of the drug to increase the surface area. These goals may be achieved by nucleating drug particles from solution in the presence of stabilizing excipients. In the spray freezing into liquid (SFL) process, a drug containing solution is atomized and frozen rapidly to engineer porous amorphous drug/excipient particles with high surface areas and dissolution rates. Aqueous suspensions of nanostructured particles may be produced from organic solutions by evaporative precipitation into aqueous solution (EPAS). The suspensions may be dried by lyophilization. The particle size and morphology may be controlled by the type and level of stabilizers. In vivo studies have shown increased bioavailability of a wide variety of drugs particles formed by SFL or EPAS. For both processes, increased serum levels of danazol (DAN) were observed in mice relative to bulk DAN and the commercial product, Danocrine®. Orally dosed itraconazole (ITZ) compositions, formed by SFL, produce higher serum levels of the drug compared to the commercial product, Sporanox® oral solution. Additionally, nebulized SFL processed ITZ particles suspended in normal saline have been dosed via the pulmonary route and led to extended survival times for mice inoculated with *Aspergillis flavus*. SFL and EPAS processes produce amorphous drug particles with increased wetting and dissolution rates, which will subsequently supersaturate biological fluids in vivo, resulting in increased drug bioavailability and efficacy.

Keywords: Spray-freezing into liquid (SFL); Evaporative precipitation into aqueous solution (EPAS); Poorly water soluble drugs; Oral delivery; Bioavailability; Pulmonary delivery

1. Introduction

Significant barriers exist for the delivery of new therapeutic compounds. Many of the new chemical entities (NCE) that are being developed are water-insoluble small molecule drugs. Many drugs are not water soluble, based on the maximum human dose, in up to 250 mL of aqueous media over the pH range 1–7.5 (Martinez et al., 2002). They make up about 40% of the new compounds in development (Fuji-Kezai, 2003). These drugs exhibit poor wetting and dissolution in the gastrointestinal tract, leading to poor absorption and poor bioavailability. Compositions containing poorly water soluble drugs prepared by cryogenic and nucleation processes have been reported (Hu et al., 2002, 2003; Rogers et al., 2001, 2002a; Chen et al., 2002; Sarkari et al., 2002). These processes are used to reduce the primary particle size of poorly water soluble drugs and form stable formulations that are readily wetted and have high dissolution rates, and in some cases high levels of supersaturation. Cryogenic processing techniques, such as spray freezing into liquid (SFL), have shown promise in the areas of oral delivery, pulmonary delivery, and nanoparticle encapsulation (Vaughn et al., 2005, 2006a; Yu et al., 2002;

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Leach et al., 2005). Cryogenic processes can offer significant improvement over other conventional processing methods such as milling, solvent evaporation, and spray-drying. Ball milling and colloid milling introduce frictional forces into the system during processing, allowing for the potential of drug degradation. Solvent evaporation and spray drying techniques require the use of scrubbers to prevent release of organic solvent into the atmosphere. High temperatures needed for the evaporation of aqueous solvents during the spray-drying process can also lead to degradation of thermally labile drugs (Overhoff et al., 2006). The surface area of powders produced by solvent evaporation and spray drying is typically below 5 m^2/g, much less than in SFL.

Several studies have reported SFL processing of poorly water soluble drugs (Hu et al., 2002, 2003; Rogers et al., 2001, 2002a). In SFL an aqueous or aqueous-organic cosolvent drug-containing solution is atomized into a cryogenic liquid, providing for ultra-rapid freezing of the drug solution. Liquid nitrogen has been employed as the cryogenic liquid due to nearly instantaneous freezing rates of the drug solutions resulting from the low boiling point of the liquid (−196 °C). The low temperatures inhibit the degradation of thermally labile drugs. The drug containing feed solution is atomized below the surface of the liquid, creating high turbulent forces and producing microparticles composed of nanostructured aggregates with high surface areas. The suspended frozen droplets can then be separated from the cryogen by allowing it to evaporate. Lyophilization of the aqueous or aqueous-organic solvents from the resulting frozen slurry produces a fine powder. SFL powders exhibit desirable properties for enhancing bioavailability such as high surface area, increased drug dissolution rates, and amorphous character. The intense atomization of the drug containing solution during processing leads to rapid nucleation and quenching of the growth of the drug and excipient particles leading to high surface area powders. As a result of ultra-rapid freezing rates, phase separation of the solutes in the drug solution is eliminated or greatly minimized, and the growing amorphous particles do not have time to crystallize. Highly porous drug/excipient aggregates are formed wherein the drug substance is molecularly dispersed in the excipients. During dissolution, media can penetrate the pores of the high surface area SFL powder, allowing for high dissolution rates of the poorly water soluble drugs (Hu et al., 2004).

Nucleation techniques to reduce the primary particle size of poorly water soluble drugs have been reported. Supersaturation of a drug solution may be achieved by various techniques including evaporation of an organic solvent or by addition of an anti-solvent. Evaporative precipitation into aqueous solution (EPAS) has been used to produce stable drug/excipient microparticles which exhibit high dissolution rates (Chen et al., 2002, 2004). In EPAS, the drug and/or stabilizer are dissolved in a water immiscible organic solvent, and an atomization nozzle is used to spray the solution into a heated aqueous solution that may contain hydrophilic stabilizers. The organic solvent is rapidly evaporated in the heated aqueous solution, providing for high supersaturation and rapid drug precipitation. The stabilizers adsorb onto the surface of the newly-formed drug particles, decreasing the interfacial tension, and allowing for particle size reduction and increased dissolution of the corresponding powders. Particles can be dried by a variety of techniques including spray-drying and ultra-rapid freezing/lyophilization (Sarkari et al., 2002). Particle size and morphology of the nucleated particles are influenced by stabilizer type and concentration in the aqueous and/or organic solution. The particle size and morphology may be controlled to achieve high dissolution rates (Sinswat et al., 2005).

The purpose of this manuscript is to summarize in vitro and in vivo studies performed with drug particles formed by the SFL and EPAS processes. SFL has been shown to increase the dissolution rates of poorly water soluble drugs such as danazol (DAN) (Hu et al., 2002), itraconazole (ITZ) (Vaughn et al., 2006a), and carbamazepine (CBZ) (Rogers et al., 2002b). Additionally, results in animals show increased bioavailability of these drugs processed by SFL (Vaughn et al., 2006a). EPAS has been used to process drugs such as carbamazepine (CBZ) (Sarkari et al., 2002) and danazol (DAN) (Chen et al., 2004), yielding rapidly dissolving particles with increased bioavailability (Vaughn et al., 2006a). The physicochemical properties of the formulations produced by SFL and EPAS, along with supporting in vivo data, show promise for their use in optimizing performance of drug delivery systems.

2. Materials and methods

Carbamazepine USP (CBZ) and danazol USP (DAN) were purchased from Spectrum Fine Chemicals (Gardena, CA). Itraconazole USP (ITZ) was purchased from Hawkins Chemical (Minneapolis, MN). Excipients used in the studies include poloxamer 407 (P407), polyvinylpyrrolidone K-15 (PVP), also purchased from Spectrum Fine Chemicals. Deoxycholic acid (DCA) was purchased from Sigma–Aldrich Chemicals (Milwaukee, WI). HPLC grade tetrahydrofuran (THF), dichloromethane (DCM), acetonitrile (ACN), and methanol (MeOH) were purchased from EM Industries, Inc. (Gibbstown, NJ). Sodium lauryl sulfate (SLS), tris(hydroxymethyl)aminomethane (TRIS), and 1N hydrochloric acid were purchased from Spectrum Chemicals.

2.1. Dissolution testing

Dissolution properties of the formulations were determined. The United States Pharmacopoeia (USP) XXIV apparatus II dissolution testing station was used (Vankel VK6010 Dissolution testing station with Vanderkamp VK650A heater/circulator; Cary, NC). Dissolution of DAN at sink conditions was performed using 900 mL of 0.75% SLS/1.21% TRIS adjusted to pH 9.0 at 37 °C. The paddle speed was 50 rpm(revolutions per minute), and samples were taken at 2, 5, 10, 20, 30, and 60 min without media replacement (Vaughn et al., 2006a).

For ITZ using the same dissolution set-up, the dissolution medium was enzyme-free simulated gastric fluid with 0.5% sodium lauryl sulfate (pH 1.2), at 37 °C. Sampling time points for the dissolution were 2, 5, 10, 20, 30, and 60 min without media replacement (Sinswat et al., 2005).

2.2. X-ray powder diffraction

X-ray diffraction (XRD) patterns were obtained using a Philips Model 1710 X-ray diffractometer (Philips Electronic Instruments, Inc., Mahwah, NJ) with Cu $K\alpha_1$ radiation at 40 kV and 20 mA. The samples were scanned from 5–45° 2θ.

2.3. Scanning electron microscopy

A Hitachi Model S-4500 field emission scanning electron microscope (SEM) (Hitachi Instruments, Irvine, CA) was used to visualize the particles and evaluate surface morphology of the particles. Samples were sputter coated with gold after being deposited on carbon tape on a microscope stage.

2.4. Oral in vivo mouse studies

Oral in vivo bioavailability studies on DAN formulations and commercial Danocrine® (Sanofi-Synthelabo, Bridgewater, NJ) were conducted using male Swiss/ICR mice with 14 mice per study arm. Dosing was carried out using oral gavage of the formulations, with 1 mL of blood withdrawn at the sampling time points, pooling two mice each per time point. Sampling time points were 0.5, 1, 2, 4, 6, 10, and 24 h after oral administration. Drug extraction from serum samples was performed and analyzed using HPLC (Vaughn et al., 2006b).

Oral in vivo studies for oral ITZ formulations and commercial Sporanox® oral solution (Janssen Pharma, Titusville, NJ) were performed on male ICR mice. The formulations as well as the commercial product were diluted to 0.96 mg ITZ/0.4 mL. Dosing of the mice was accomplished with 0.4 mL of diluted ITZ using oral gavage. Twelve hours after the last dosing on days 3, 8 and 12, mice were sacrificed using CO_2 narcosis with blood collected by cardiac puncture and serum extraction performed to obtain ITZ. Additionally, surgery was performed to extract mouse lung tissue which was then homogenized in 1 mL normal saline. Serum and lung tissue concentrations of ITZ were measured using reverse phase HPLC (Vaughn et al., 2006b). All animal studies involving oral ITZ dosing were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

2.5. Pulmonary in vivo mouse studies

Pulmonary in vivo studies on nebulized dispersions in normal saline of ITZ formulations (20 mg/mL) were performed using male Sprague–Dawley ICR mice. The small animal dosing chamber described in McConville et al. (2005) was used to dose the mice. The chamber consists of a polymethylmethacrylate airtight box with dimensions of 40.6 cm × 11.4 cm × 21.6 cm. In accordance with the University of Texas at Austin and University of Texas Health Science Center at San Antonio Institutional Animal and Care Use Committee guidelines, an animal roam area of about 63 cm^2 was designed to hold up to 14 mice. An Aeroneb Pro micro pump nebulizer (Aerogen, Inc., Mountain View, CA) was used to nebulize the aliquot of 8 mL of dispersion into the chamber over a 20 min dosing period. Two mice were sacrificed at each time point by CO_2 narcosis (0.5, 1, 2, 4, 6, 10, and 24 h after dosing), blood was collected by cardiac puncture and serum extraction was used to obtain ITZ. Additionally, surgery was performed to extract mouse lung tissue which was then homogenized in 1 mL normal saline. Serum and lung tissue concentrations of ITZ were measured using reverse phase HPLC (Vaughn et al., 2006a).

SFL and EPAS processed ITZ were evaluated for its effectiveness against *Aspergillus flavus*, the fungus that causes systemic aspergillosis. Ten immunocompromised male ICR mice per group received either itraconazole by oral gavage (Sporanox® Oral Solution) or by nebulization (EPAS-ITZ and SFL-ITZ). Pre-emptive ITZ dosing was begun 1 day before pulmonary inoculation with *A. flavus* and continued for 12 days in the survival arm. Lungs were harvested for analysis of fungal burden by CFU enumeration 1 day after discontinuation of therapy. Mice were monitored over a 10-day period following discontinuation of therapy, and survival was assessed by Kaplan–Meier analysis. ITZ at steady-state serum and lung tissue concentrations were determined by HPLC assay (Hoeben et al., 2006). All animal studies were conducted in accordance with the IACUC guidelines.

2.6. Pharmacokinetic and statistical analysis

Pharmacokinetic and statistical analyses were performed on the data obtained from the in vivo studies. ITZ lung tissue concentration versus time was evaluated using a non-compartmental model, while ITZ serum concentration versus time was evaluated using one-compartmental analysis from extravascular administration, both using Win-NonLin version 4.1. One-way analysis of variance (ANOVA) was used to determine statistical differences between in vivo results. Results with p-values of <0.05 were considered statistically significant (Vaughn et al., 2006a; Hoeben et al., 2006).

3. Results and discussion

SFL particle engineering technology has been shown to micronize a wide range of API's for the purpose of enhancing the dissolution rate of poorly water soluble drugs. Powders produced by SFL have high dissolution rates, owing to their amorphous character (Hu et al., 2002; Rogers et al., 2002a) (data not shown). XRD of different EPAS formulations, however, shows crystalline as well as amorphous character, depending on the model drug, its concentration, and stabilizers used. In contrast to SFL processed CBZ, slowly frozen control samples of CBZ displayed peaks characteristic of crystalline CBZ. Freezing rate, therefore, has a direct impact on the formation of crystalline CBZ. Since the SFL feed solution is atomized directly into liquid nitrogen, there is not sufficient time for the CBZ molecules to crystallize prior to freezing. Slowly frozen CBZ samples, however, allow for the organization of the CBZ molecules into their thermodynamically favored crystalline habit. The organized crystalline lattice structure formed during crystallization of the slowly frozen formulations must be broken in order for dissolution to occur, whereas amorphous formulations have a higher chemical poten-

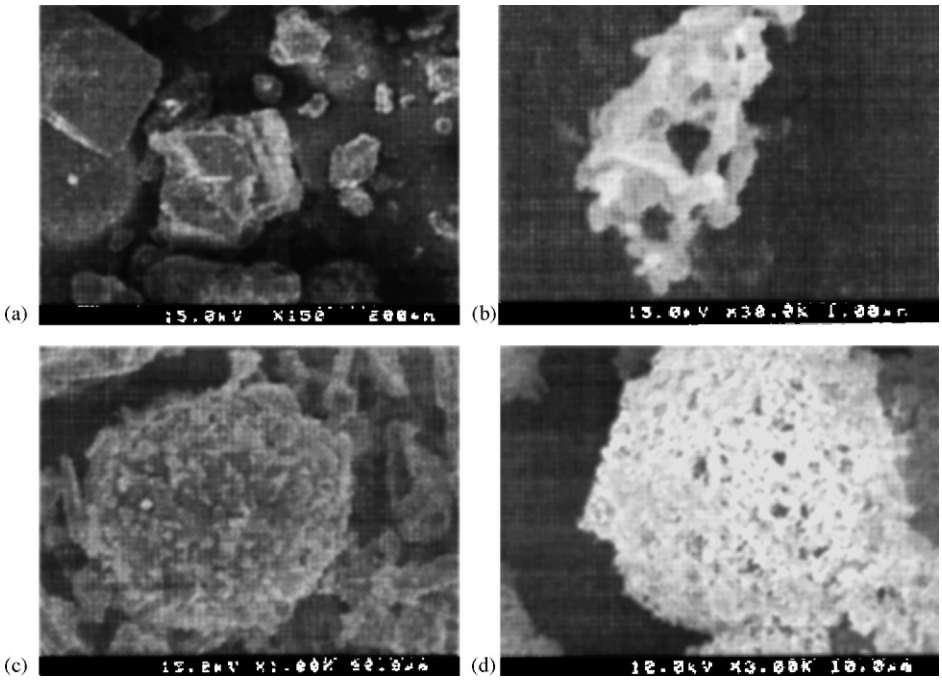

Fig. 1. SEM micrographs of (a) the physical mixture containing CBZ and excipients, (b) the SFL micronized CBZ powder, (c) the physical mixture containing danazol (DAN) and excipients, (d) the SFL micronized DAN powder (reprinted with permission from Rogers et al., 2002a).

tial and thus may form supersaturated solutions (Overhoff et al., 2006). Conversely, during a nucleation process like EPAS, drug molecules have sufficient time to organize themselves in solution before solvent evaporation and subsequent drug supersaturation, allowing for crystallization in most cases. Depending on the stabilizer type and concentration in the organic or aqueous media, crystallization of the drug may not take place, yielding amorphous or crystalline particles (Sinswat et al., 2005).

SEM micrographs of CBZ and DAN processed by SFL are shown in Fig. 1 (Rogers et al., 2002a). SFL micronized powders generally show porous particles of varying particle sizes and morphology. Physical mixtures (Fig. 1C) show bulk danazol adhering to the surface of poloxamer particles, with clear distinctions made between the drug and excipient in terms of morphology. The SFL particles, in contrast, are composed of primary drug/excipient particles of submicron size which are aggregated to form larger particles that form nanostructured aggregates.

SEM micrographs of CBZ processed by EPAS are shown in Fig. 2 (Sarkari et al., 2002). The EPAS particles show variable morphology, depending on the stabilizer used (Sinswat et al., 2005), its concentration, the dissolving solvent, and the drying method used to produce the EPAS powder (Sarkari et al., 2002). In all cases, however, SFL and EPAS formulations show increased dissolution and wetting compared with physical mixtures of the drug and excipient(s). Vaughn et al. processed danazol using EPAS and SFL, and determined its dissolution rate. The dissolution profile of DAN formulations is shown in Fig. 3 (Vaughn et al., 2006a). Within the first 2 min, the dissolution of DAN was nearly 100% for the SFL powder, compared to 80% for the EPAS powder and 40% for the bulk DAN. Within 10 min, the SFL and EPAS dissolution rates were statistically similar, and much higher than that of bulk DAN.

Dissolution of DAN at supersaturated conditions was also conducted. Supersaturated solutions of DAN formulations, as well as the contents of the commercial Danocrine® capsule, are depicted in Fig. 4 (Vaughn et al., 2006b). The SFL composition displayed 33% supersaturation compared to the physical mixture, after 90 min. The EPAS composition supersaturated the dissolution medium 27% above the control (physical mixture). Addition of PVP to the physical mixture allowed DAN to achieve a higher apparent solubility than the Danocrine® capsule powder. All of these apparent solubility values are above the equilibrium solubility of crystalline DAN which is 0.47 mg/mL.

The ability of these formulations to supersaturate the dissolution media highlights the possibility for increased bioavailability when administering the amorphous drug form. The meta-stable solubility of an amorphous drug form may be as high as 100-times greater than the equilibrium solubility of the crystalline form (Hancock and Zograffi, 1997; Hancock and Parks, 2000). If the concentration of drug in solution is significantly increased, the higher chemical potential will lead to an increase in flux across an exposed membrane (Raghavan, 2000). This may lead to much higher blood levels for an amorphous drug form, compared to an identical crystalline formulation. Thus high concentration levels in the gastrointestinal tract have the potential to lead to an improvement in oral bioavailability.

Aqueous dissolution rates have been correlated to the in vivo performance of drug delivery systems, however, modeling of these systems have proven difficult and variable. In vivo testing of these delivery systems, therefore, proves invaluable in determining the performance of drug formulations. Drug formu-

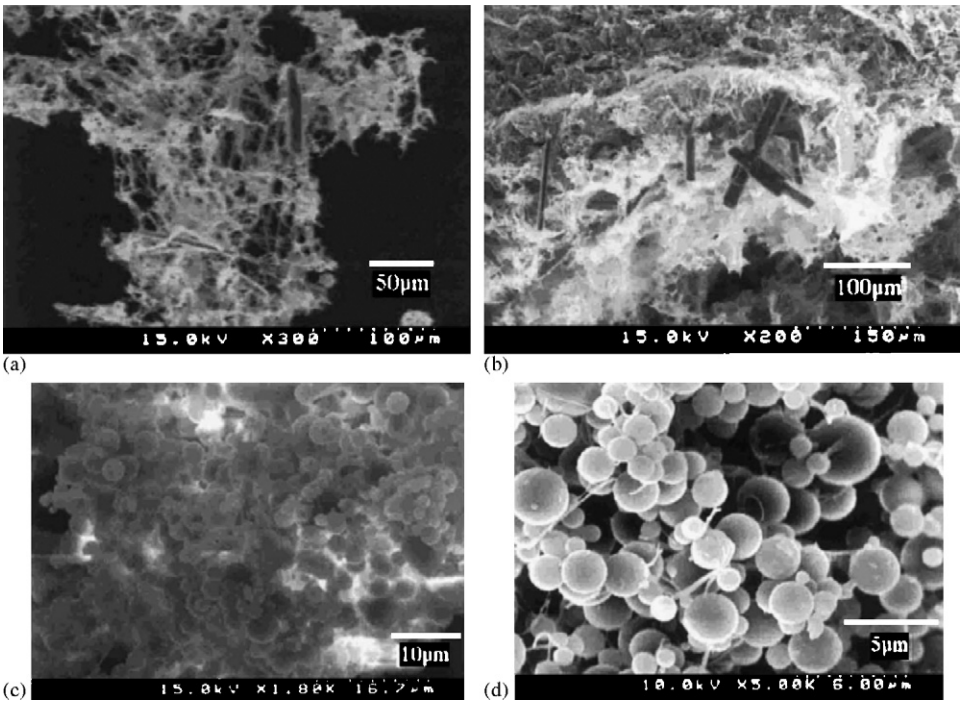

Fig. 2. SEM images of EPAS processed and spray dried and ultra-rapid frozen CBZ. (a) CBZ:P407:DCA (1:0.5:1.08) drying technique: ultra-rapid freezing (b) CBZ:P407:PVP (1:0.5:1.08) drying technique: ultra-rapid freezing (c) CBZ:PF407:DCA (1:0.5:0.5) drying technique: spray drying (d) CBZ:DCA (1:0.76) drying technique: spray drying (reprinted with permission from Sarkari et al., 2002).

lations produced by SFL and EPAS have been studied in vivo, for their delivery via the pulmonary and oral routes (Vaughn et al., 2006a,b; Hoeben et al., 2006). Oral bioavailability of SFL and EPAS DAN formulations administered to mice is depicted in Fig. 5 (Vaughn et al., 2006b) and Table 1 (Vaughn et al., 2006a) shows the corresponding pharmacokinetic parameters. AUC values for DAN processed using SFL and EPAS were determined to be 2626 and 1613 ng h/mL, respectively, compared to 1648 ng h/mL for the Danocrine® capsules, and 703 ng h/mL for the bulk DAN. C_{max} values for the SFL and EPAS DAN were calculated to be 392 and 430 ng/mL, respectively, while the value for Danocrine® capsules was 199 and 204 ng/mL for the bulk DAN. Increased AUC and Cmax values indicate higher bioavailability of DAN from EPAS and SFL formulations compared to commercial Danocrine® and bulk DAN. This increased bioavailability may be attributed to the increased dissolution rate and higher supersaturation of these formulations in the gastrointestinal tract. The comparison of the in vivo data to the in vitro supersaturation data indicates that formulations which supersaturate the dissolution medium enhance flux across the intestinal membrane, giving rise to higher systemic concentrations of ITZ.

Itraconazole bioavailability from pulmonary administration was evaluated using a mouse model. The pulmonary dosing of the animals was carried out using the small animal pulmonary

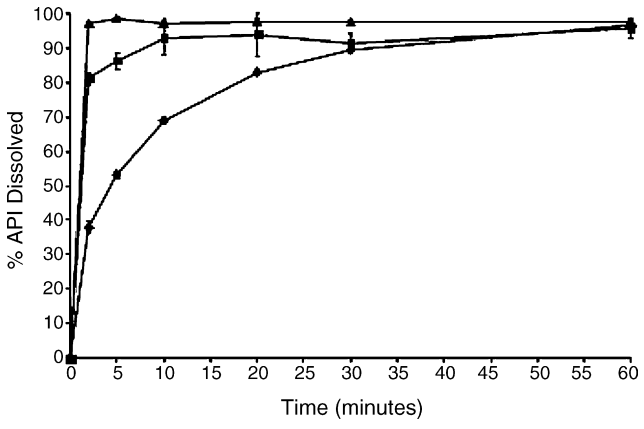

Fig. 3. Dissolution profile for SFL (▲) and EPAS (■) processed DAN compared to bulk DAN (♦) (USP II, 50 RPM, 0.5% SLS pH 9 Tris buffer $n = 6$) (reprinted with permission from Vaughn et al., 2005).

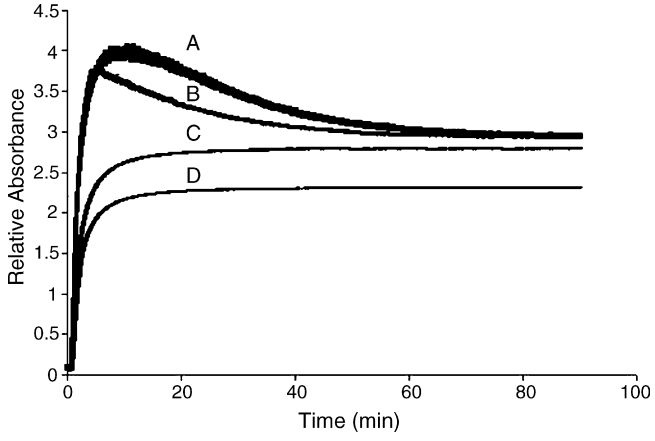

Fig. 4. Supersaturated dissolution in 0.75% SDS 1.21% Tris buffer at pH 9 for (A.) the DAN SFL composition (DAN:PVP 1:1), (B) the DAN EPAS composition (DAN:PVP 1:1), (C) physical mixture (DAN:PVP 1:1), and (D) Danocrine® capsule contents (reprinted with permission from Vaughn et al., 2006a).

Table 1
Pharmacokinetic parameters calculated using non-compartmental analysis in Win-Nonlin of the mice dosed with EPAS and SFL compositions, physical mixture, and Danocrine® capsule powder

ITZ Formulation	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	K_{el} (h^{-1})	AUC (0–24) (ng h/mL)	AUC_{inf} (ng h/mL)
Danocrine® capsules	199.3	1.0	6.7	0.103	1519	1648
Physical mixture (danazol:PVP-K15 1:1)	204.4	1.0	4.5	0.154	672	703
EPAS composition (danazol:PVP-K15 1:1)	430.1	1.0	4.6	0.150	1534	1613
SFL composition (danazol:PVP-K15 1:1)	392.5	1.0	4.4	0.158	2558	2626

Reprinted with permission from Vaughn et al., 2006a.

dosing chamber described previously (McConville et al., 2005). Fig. 6 (Vaughn et al., 2006a) shows the uniformity of ITZ pulmonary dosing for each of the animals tested. The dose uniformity and amount of ITZ received per animal is in agreement with previous studies demonstrating uniform pulmonary dosing inside the chamber (McConville et al., 2005). Average lung tissue concentrations of ITZ pulmonary formulations are shown in Fig. 7 (Vaughn et al., 2006b). The average lung tissue concentrations (μg/g wet lung tissue) for ITZ pulmonary and Sporanox® oral solution on day 3 were 2.16 and 0.19; on day 8, they were 2.22 and 0.15; and on day 12, they were 2.52 and 0.18, respectively. ITZ-pulmonary achieved significantly greater lung tissue concentrations compared to Sporanox® oral solution. Average blood serum concentrations of ITZ pulmonary formulations are shown in Fig. 8 (Vaughn et al., 2006b). Table 2 (Vaughn et al., 2006b) shows the pharmacokinetic parameters derived from the in vivo bioavailability data from the mouse.

Oral and pulmonary delivery of drugs offers non-invasive routes of administration with better patient compliance than parenteral administration. Inhalation therapy using antifungal agents such as ITZ can be utilized both to treat and prevent systemic fungal infection. Combination treatments involving oral and inhalation therapy may also prove beneficial to these patients (Steinbach, 2005). Prophylactic therapy with antifungal treatment is recommended for at risk patients prior to surgical

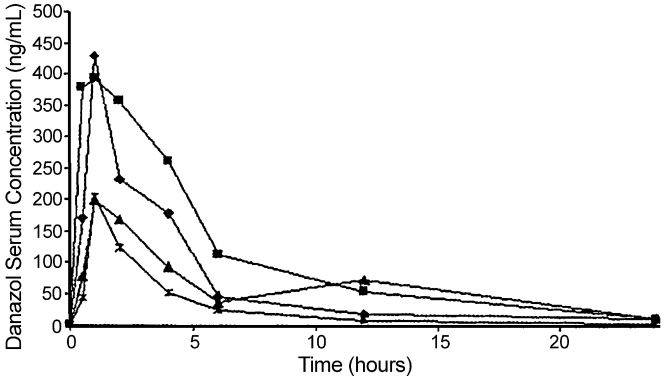

Fig. 5. Oral bioavailability of danazol in a mouse model for (■) the SFL composition DAN:PVP (1:1), (♦) the EPAS composition DAN:PVP (1:1), (*) the physical mixture DAN:PVP (1:1), and (▲) the Danocrine® capsule contents (reprinted with permission from Vaughn et al., 2006b).

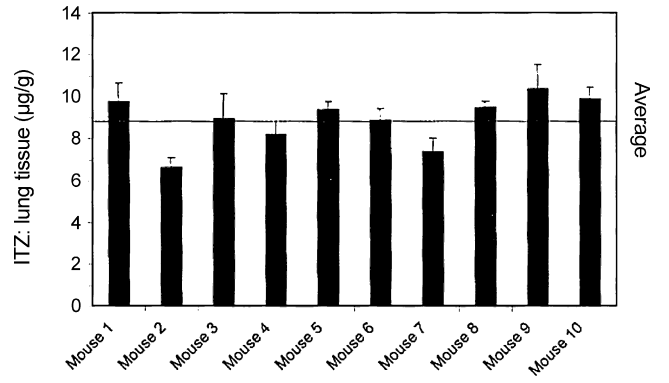

Fig. 6. Dose uniformity based on individual wet lung weights for mice dosed with an amorphous ITZ pulmonary composition (reprinted with permission from Vaughn et al., 2006b).

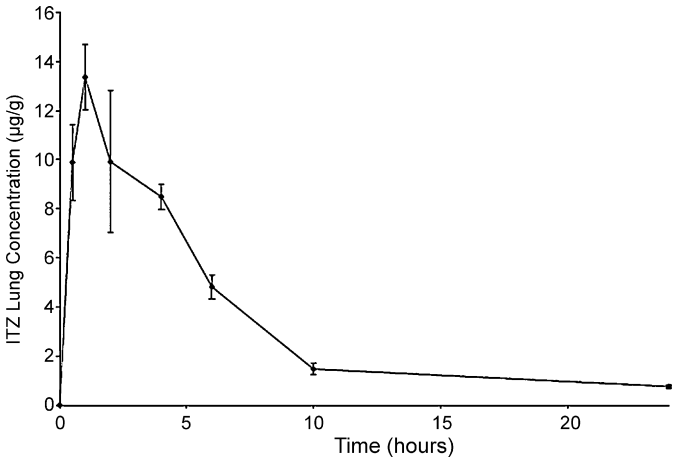

Fig. 7. Average ITZ lung tissue concentrations in mice dosed with ITZ-pulmonary. $N = 2$ mice per time point with four individual extractions from each mouse (reprinted with permission from Vaughn et al., 2006b).

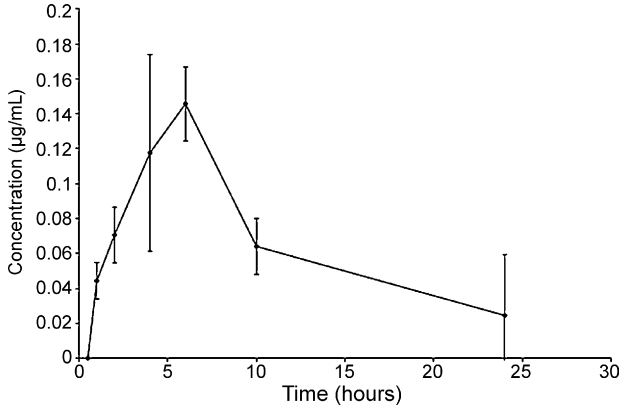

Fig. 8. Average ITZ serum concentrations over a 24 h period for mice dosed with ITZ-pulmonary. $N = 2$ mice per time point (reprinted with permission from Vaughn et al., 2006b).

Table 2
Pharmacokinetic parameters for lung and serum concentrations from mice dosed with amorphous ITZ pulmonary composition

Pharmacokinetic parameter	Lung[a]	Serum[b]
C_{max} (μg/g)	13.4	0.12
T_{max} (h)	1	5.35
$T_{1/2\,K_{01}}$ (h)		3.73
$T_{1/2\,K_{10}}$ (h)	5.5	3.70
K_{01} (h^{-1}) absorption		0.186
K_{10} (h^{-1}) elimination	0.13	0.188
AUC_{inf} (μg h/mL)	85.8	1.69

[a] Based on non-compartmental analysis of the lung tissue concentrations vs. time.
[b] Calculated based on one-compartment analysis of the serum concentrations vs. time for extravascular administration (reprinted with permission from Vaughn et al., 2006b).

procedures, and pre-emptive antifungal therapy is recommended for patients with bronchial airway abnormalities and persistent *Aspergillus* colonization (Sole et al., 2005). SFL and EPAS processed ITZ has been used prophylactically to extend survival of mice that have been inoculated with *A. flavus*, showing improved efficacy over conventional treatment using Sporanox® Oral Solution (Hoeben et al., 2006). Hoeben et al. (2006) processed itraconazole by SFL and EPAS, and evaluated the formulations for effectiveness against the fungus *A. flavus*, the cause of systemic fungal infections in AIDS patients and others who are immunocompromised. Mice were inoculated with *Aspergillus* after dosing with a prophylactic amount of ITZ or a control. Immunosuppression and subsequent inoculation of the animals with *Aspergillus* provides for fungal infection which quickly becomes systemic in nature. Aerosolized EPAS and SFL formulations of ITZ provided a significant survival benefit as preventive therapy. Significant differences in survival were shown between these formulations compared to Sporanox® oral solution and controls. Survival curves from the pulmonary dosed ITZ are shown in Fig. 9 (Hoeben et al., 2006). The SFL formulation of ITZ had the longest median survival (> 20 days) and was significantly greater than that of the control (median survival 5 days; $p < 0.005$) and Sporanox® (median survival 4 days; $p < 0.001$). The EPAS formulation of ITZ (median survival 11 days) also demonstrated a survival advantage over Sporanox® ($p = 0.04$). No differences in survival rates were observed between aerosolized EPAS and SFL formulations. Despite adequate peak serum concentrations, orally administered Sporanox® Oral Solution conferred no survival advantage compared to controls. Results, therefore, show efficacy of the pulmonary dosed SFL and EPAS ITZ against the *Aspergillus* fungus in vivo, exhibiting greater antifungal effectiveness at the pulmonary site of infection than the commercial product (Hoeben et al., 2006).

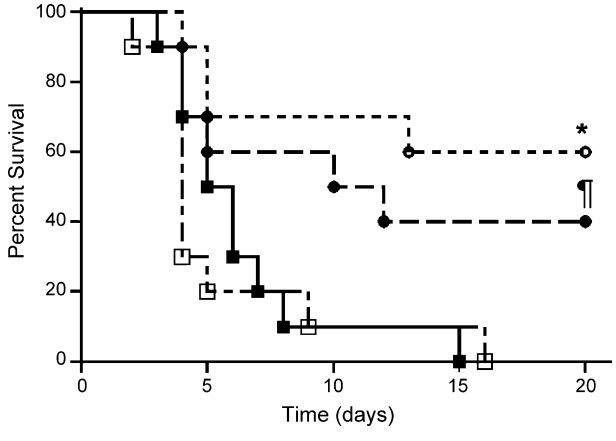

Fig. 9. Survival curves for mice ($n = 10$ per group) that received prophylaxis with aerosolized itraconazole prepared by evaporative precipitation of aqueous solution (EPAS, ●) and spray-freeze into liquid (SFL, ◯), or orally administered Sporanox Oral Liquid (SOL, □) formulations of itraconazole and controls (■), and challenged with *A. flavus*. $N = 10$ mice per group. *$p < 0.005$ vs. controls and $p < 0.001$ vs. SOL. $p = 0.06$ vs. controls and $p = 0.04$ vs. SOL (reprinted with permission from Hoeben et al., 2006).

4. Conclusion

The SFL and EPAS particle engineering technologies are used to produce rapidly dissolving formulations of poorly water soluble drugs. The high dissolution rates of these formulations have been shown to produce increased oral bioavailability in vivo. Pharmacokinetic analysis of oral in vivo data using DAN showed higher AUC values than the commercial product or bulk crystalline DAN. Nebulized SFL and EPAS formulations of ITZ exhibited high bioavailability and high efficacy against fungal infections in vivo, allowing for extended survival rates of animals. SFL and EPAS technologies, therefore, have the potential for use in producing a variety of effective formulations for human treatment, both orally and pulmonarily.

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