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Research paper

Single dose and multiple dose studies of itraconazole nanoparticles

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Abstract

The objective of this study was to determine and compare the lung and serum concentrations in mice following oral and pulmonary dosing of amorphous nanoparticulate itraconazole (ITZ) compositions as well as the Sporanox® oral solution (itraconazole/Janssen). Second, the steady state partitioning of ITZ in lung tissue and circulatory compartments following repeated oral and pulmonary dosing was determined. The pulmonary formulation (ITZ-pulmonary) consisted of ITZ, polysorbate 80, and poloxamer 407 in a 1:0.75:0.75 ratio and the oral formulation (ITZ-oral) consisted of ITZ, PEG 8000, poloxamer 188, and sorbitan monooleate 80 in a 1:1:2:1 ratio. Mice were dosed every 12 h by nebulization with ITZ-pulmonary, or by oral gavage with ITZ-oral or Sporanox oral solution ($n = 12$ per study arm). ITZ-pulmonary achieved significantly greater (>10-fold) lung tissue concentrations compared to the Sporanox oral solution and ITZ-oral. There were no statistical differences between the two oral formulations. ITZ-pulmonary achieved significantly greater lung levels per unit serum concentration compared to the orally dosed ITZ compositions. High and sustained lung tissue concentrations were achieved via inhalation of an amorphous nanoparticulate ITZ-pulmonary composition while maintaining serum levels which are above the minimum lethal concentration (MLC) of *Aspergillus fumigatus*.

Keywords: Itraconazole; Nanoparticles; Spray freezing into liquid; Amorphous; Pharmacokinetics; Lung deposition

1. Introduction

With an ever-increasing population of patients at risk for invasive fungal infections, the incidence of these infections has increased dramatically over the past 20 years. Much of the dramatic increase is due to proliferation of diseases and procedures which render patients in an immunocompromised state. These populations include patients with acquired immunodeficiency syndrome (AIDS), patients who have undergone solid organ or hematopoietic stem cell transplants (HSCT), individuals receiving immunosuppressant therapy, and patients receiving aggressive chemotherapy for malignancies. *Aspergillus* spores can be found airborne or on many hospital surfaces. It has been associated with an increasing incidence among immunocompromised patients resulting in very high mortality rates [1,2]. The portal of entry is typically the lungs where the fungi will present as pneumonia, cavitary infiltrates or nodules [3]. Dissemination of the *Aspergillus* infection is typically through the lymph system and can involve organs such as the heart, kidney, central nervous system, gastrointestinal tract, spleen, liver, thyroid gland, and pancreas [1]. Mortality rates for *Aspergillus* infections are nearly always fatal in disseminated disease with up to 90% mortality rates among central nervous system (CNS) patients [4].

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The first line treatment for invasive aspergillosis is the polyene amphotericin B deoxycholate [5]. It shows extensive infusion related and liver toxicity in many patients. For this reason, liposomal and colloidal lipid dispersions were developed and showed much lower toxicities, although not significantly improving mortality rates [6]. It has been reported that amphotericin B and liposomal amphotericin B formulations have been administered through nebulization to treat and prevent pulmonary fungal infections in patients with high risk of infection [7–10]. Prophylaxis with amphotericin B inhalation was unsuccessful in patients with prolonged neutropenia [11].

ITZ is a poorly water-soluble active pharmaceutical ingredient (API) which displays low solubility and pH dependent dissolution which causes low and variable oral absorption. Absolute bioavailability of the oral capsule is 55% in the fed state and 40% lower in the non-fed state [12]. Following intravenous administration the volume of distribution is 10.7 L/kg and it is 99.8% protein bound [13]. Tissue distribution is high in the nails, skin, muscle, and liver, whereas, distribution to the cerebral spinal fluid, eye, saliva, and sputum is negligible [14]. Improvement of ITZ therapy through particle engineering processes and novel delivery mechanisms has been reported [15]. Lung concentrations of ITZ resulting from oral or intravenous administration have been reported in mice [16] and in humans [17]. In those references, lung:serum ratios for the orally administered Sporanox oral solution differ from the levels found in this study. In mice [16], the lung:serum ratio was much lower than what was found in this study, whereas, in humans [17] the ratio was much higher. Although, lung:serum concentration ratios have not been reported for ITZ dosed via inhalation. ITZ-pulmonary administration has shown significant improvements in survival of a murine infection model compared to the Sporanox oral solution [18].

Formation of amorphous ITZ nanoparticles can be achieved via SFL technology. In this particle engineering process, a feed solution containing an API and dissolution enhancing excipient(s) is atomized directly into a cryogenic liquid, such as nitrogen. The resulting dried powder is composed of discrete microparticles where the API is molecularly dispersed with a polymer in a porous matrix [19]. This molecular dispersion is achieved by rapid freezing in liquid nitrogen, which prevents phase separation. In previous studies, it was found that enhanced dissolution is due to the amorphous morphology, high surface area, and enhanced wettability of the SFL nanostructured particles [20].

The objective of this study was to determine the lung and serum concentrations in mice following pulmonary dosing of amorphous nanoparticles of ITZ over a 24 h period. The hypothesis is that sufficient serum concentrations and high lung concentrations are achieved through pulmonary dosing of amorphous ITZ nanoparticles. The serum pharmacokinetics of the inhaled ITZ nanoparticles were evaluated using a one compartment model for extravascular administration. Also, the pharmacokinetic parameters for ITZ in lung tissue over a 24 h period were calculated using non-compartmental analysis. Lastly, the steady state partitioning of ITZ in lung tissue and serum following repeated oral and pulmonary dosing was evaluated.

2. Materials and methods

2.1. Materials

The following materials were purchased: itraconazole USP (ITZ; Hawkins Chemical, Minneapolis, MN); ketoconazole USP, poloxamer 407, polysorbate 80, poloxamer 188, sorbitan monooleate 80, polyethylene glycol (PEG) 8000, potassium phosphate monobasic, sodium hydroxide (NaOH), and sodium chloride (NaCl) (Spectrum Chemicals, Gardena, CA); high-performance liquid chromatography (HPLC) grade acetonitrile and dichloromethane (EM Industries, Inc., Gibbstown, NJ). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ).

2.2. Production of ITZ nanoparticles

Amorphous nanoparticulate ITZ compositions (ITZ-pulmonary and ITZ-oral) were produced using the particle engineering technology spray freezing into liquid (SFL) [21]. ITZ-pulmonary consisted of ITZ, polysorbate 80, and poloxamer 407 in a 1:0.75:0.75 ratio and the ITZ-oral consisted of ITZ, PEG 8000, poloxamer 188, and sorbitan monooleate 80 in a 1:1:2:1 ratio. ITZ and excipients were dissolved in an acetonitrile/dichloromethane co-solvent system and atomized through a 63 μm poly-ether-ether-ketone (PEEK) nozzle (Upchurch Scientific, Oak Harbor, WA) via an HPLC pump (Jasco PU-2086 plus, Jasco Inc., Easton, MD) at 20 mL/min below the surface of liquid nitrogen. The frozen nanostructured aggregates were then separated from the liquid nitrogen and the solvent was removed by lyophilization (VirTis Advantage, VirTis, Gardiner, NY). The dried powders were stored under vacuum until administration.

2.3. Pulmonary dosing of a murine model

Twenty-six male Harlan–Spague–Dawley ICR mice (Hsd:ICR, Harlan Sprague Dawley, Inc., Indianapolis, IN) were dosed with ITZ-pulmonary using the dosing chamber described previously [22]. Briefly, the chamber consisted of a polymethylmethacrylate (PMMA) airtight box ($40.6 \times 11.4 \times 21.6$ cm) with a hinged top, having a nominal wall thickness of 1.25 cm. The chamber was designed to hold up to 14 mice, each having a floor area of approximately 63 cm^2, in accordance with The University of Texas at Austin and the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee (IACUC) guidelines. A 20 mg/mL ITZ-pulmonary dispersion was formed in 4 mL of normal saline by first wetting the powder followed by sonication

for 1 min prior to dosing. An Aeroneb® Pro micro pump nebulizer (Aerogen, Inc., Mountain View, CA) was situated at the inlet of the chamber and nebulization of 8 mL dispersions was conducted over 20 min for each dose. For the 24 h pharmacokinetic study, two mice were sacrificed by carbon dioxide narcosis at each time point (0.5, 1, 2, 4, 6, 10, and 24 h). Serum was collected, lungs were extracted and both were analyzed for ITZ content. Dosing was conducted every 12 h through the completion of the study. Twelve hours after the last dose (trough levels) on days 3, 8, and 12, four mice were sacrificed by carbon dioxide narcosis. Blood was collected by cardiac puncture, allowed to clot for 20 min, centrifuged, and serum was collected. Surgery was performed on each mouse to extract the lung tissue which was then homogenized in 1 mL of normal saline and four 0.25 mL aliquots were analyzed for ITZ by reverse-phase high-performance liquid chromatography (HPLC).

2.4. Oral dosing of a murine model

Male ICR mice were dosed with either ITZ-oral or Sporanox oral solution by oral gavage twice daily through the completion of the study. The ITZ-oral composition was prepared by dispersing ITZ-oral (0.96 mg ITZ/0.4 mL) in deionized water followed by sonication for 1 min. The Sporanox oral solution was diluted to 0.96 mg/0.4 mL) with United States Pharmacopoeia (USP) phosphate buffer at pH 1.7. The mice were dosed with 0.4 mL of the dispersion by oral gavage. Twelve hours after the last dose on days 3, 8, and 12, four mice from each group were sacrificed by carbon dioxide narcosis. Blood was collected by cardiac puncture, allowed to clot for 20 min, centrifuged, and serum was collected. Surgery was performed on each mouse to extract the lung tissue, which was then homogenized in 1 mL of normal saline and separated into four 0.25 mL aliquots.

2.5. Serum and lung extraction and chromatographic analysis

Calibration standards, serum, and homogenized lung samples were analyzed using a method previously published [23]. Briefly, normal saline (1 mL) was added to each harvested lung sample, which was then homogenized using tip ultrasonication. Aliquots of homogenate (250 μL) were transferred to 4 separate vials and drug was extracted similarly to the serum samples. Barium hydroxide 0.3 N (50 μL) and 0.4 N zinc sulfate heptahydrate solution (50 μL) were then added to each to precipitate out water-soluble proteins. The samples were then vortex mixed (30 s). Acetonitrile (1 mL) containing 100 ng/mL ketoconazole as an internal standard was added before a further vortex mixing (1.5 min), followed by centrifugation at 3000*g* (15 min). The supernatant was transferred to 1.5 mL centrifuge tubes and seated in an aluminum heating block (60 °C), under a stream of nitrogen for 1 h until dry.

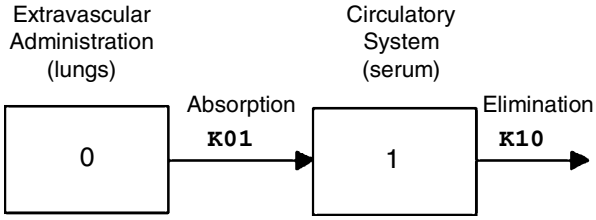

Fig. 1. One-compartment pharmacokinetic model used to evaluate the serum concentrations measured from mice dosed with the ITZ-pulmonary and to estimate pharmacokinetic parameters.

Samples were then reconstituted with 250 μL mobile phase (62% acetonitrile: 38% of 0.05 M potassium phosphate monobasic buffer adjusted to pH 6.7 with NaOH) and vortex mixed (1 min) before filtering (0.45 μm) into HPLC glass injection vials with low volume inserts (150 μL). Each sample was analyzed using a Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Columbia, Maryland) equipped with a heated (37 °C) C-18 base-deactivated column (5 μm, 250 × 4.6 mm) protected by a C-18 guard column (5 μm, 7.5 × 4.6 mm) (Alltech Associates, Inc., Deerfield, IL). The mobile phase eluted an ITZ peak at 17.7 min and ketoconazole at 7.3 min at a flow rate of 1.0 mL/min, an injection volume of 100 μL, and an absorption wavelength of 263 nm (λ_{max}). The limit of detection for ITZ was 10 ng/mL and the limit of quantitation was 30 ng/mL.

2.6. Pharmacokinetic analysis

Mice dosed with the ITZ-pulmonary composition were evaluated for 24 h lung and serum pharmacokinetics. The lung tissue concentration vs time was evaluated using a non-compartmental model in WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA). The serum concentration vs time was evaluated using a one-compartmental analysis for extravascular administration using Win-NonLin. Graphing of the serum data on a semi-log plot revealed a first-order absorption phase and a first-order elimination phase. For this reason, a one-compartment model was constructed and estimations of the pharmacokinetic parameters were calculated. Fig. 1 illustrates the model used and the parameters which were calculated.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine statistically significant differences between results. Results with p-values < 0.05 were considered statistically significant.

3. Results

3.1. Single dose, 24 h pharmacokinetics

The one-compartment model, which was utilized for analysis of the serum concentrations, is illustrated in

Fig. 1. Concentration vs time for the lung tissue and serum are shown in Figs. 2 and 3. From the figures and Table 1, the lung tissue C_{max}, T_{max}, $T_{1/2}$, K_{10}, and AUC_{inf} were 13.4 μg/g, 1 h, 5.5 h, 0.13 h^{-1}, and 85.8 μg h/mL, respectively. Based on a one-compartmental analysis as shown in Fig. 1, the predicted serum C_{max}, T_{max}, $T_{1/2\ K01}$, $T_{1/2\ K10}$, K_{01}, K_{10}, and AUC_{inf} were 0.12 μg/mL, 5.35 h, 3.73 h, 3.7 h, 0.186 h^{-1}, 0.188 h^{-1}, and 1.69 μg h/mL.

3.2. Morphological observations in repeat dose groups

Over the 12 day dosing period, morphological observations of the morbidity and mortality of the mice were noted and are shown in Table 2. Two deaths associated with toxicity to the Sporanox oral solution occurred on day 7 of dosing. Poor skin turgor was noted in the Sporanox oral solution group which is a sign of dehydration associated with diarrhea, which was also observed in that entire group. The grooming practices in the Sporanox oral solution group began to decline following multiple day dosing (>7 days) which was indicated by unkempt fur. Furthermore, the mice in the Sporanox oral solution group were highly resistant to dosing, due to the taste, or low pH of the solution, or some other unmeasured property of the oral solution. None of these symptoms were noted in the amorphous ITZ groups,

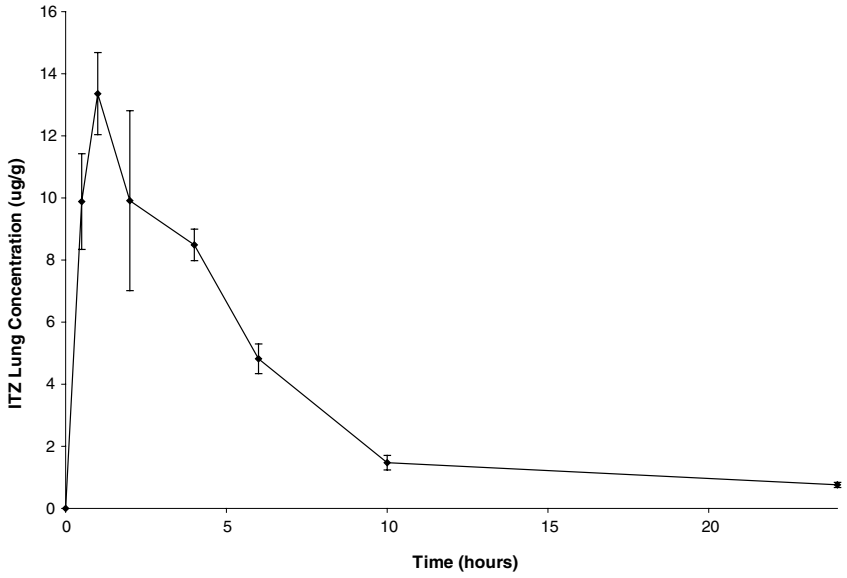

Fig. 2. Average ITZ lung tissue concentrations in mice dosed with ITZ-pulmonary. $N = 2$ mice per time point with four individual extractions from each mouse.

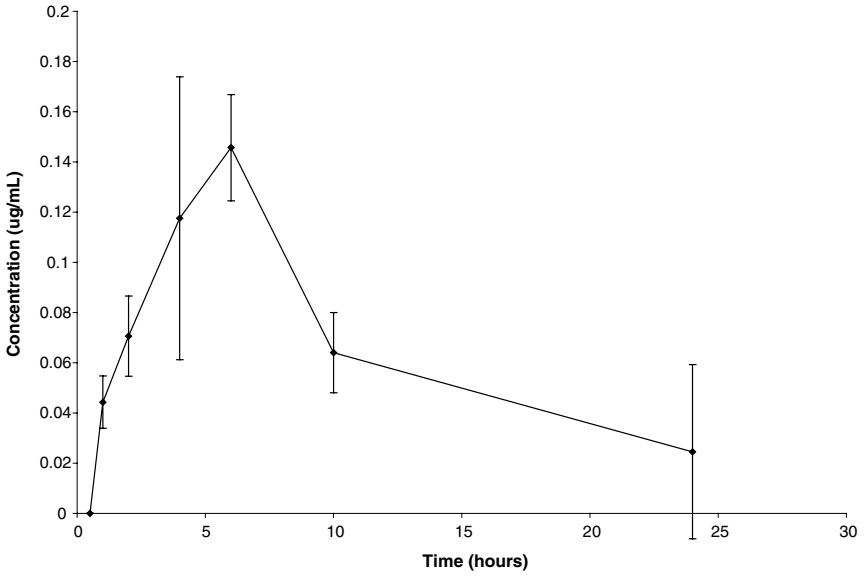

Fig. 3. Average ITZ serum concentrations over a 24 h period for mice dosed with ITZ-pulmonary. $N = 2$ mice per time point.

Table 1
Pharmacokinetic parameters for lung and serum concentrations from mice dosed with the amorphous ITZ-pulmonary composition

Pharmacokinetic parameter	Lung[a]	Serum[b]
C_{max} (μg/g)	13.4	0.12
T_{max} (h)	1	5.35
$T_{1/2\ K01}$ (h)		3.73
$T_{1/2\ K10}$ (h)	5.5	3.70
K_{01} absorption (h^{-1})		0.186
K_{10} elimination (h^{-1})	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-compartmental analysis of the serum concentrations vs time for extravascular administration.

Table 2
Morphological observations in mice dosed with ITZ-pulmonary, ITZ-oral, and with the Sporanox® oral solution; (+) symptoms were observed in mice from that group; (−) no symptoms were observed

	ITZ-pulmonary	Sporanox® oral solution	ITZ-oral
Dose related deaths[a]	0	2	0
Evidence of dehydration[b]	−	+	−
Diarrhea[c]	−	+	−
Decreased grooming[d]	−	+	−
Dosing resistance[e]	−	+	−

[a] Indicates the total number of deaths during the study period.
[b] Mice displayed poor skin turgor upon scruffing during dosing and were lethargic.
[c] Diarrhea was evident by moist and watery stool.
[d] Decreased grooming was noted as fur which was unkempt and soiled.
[e] Immediate resistance to dosing upon insertion of the gavage tip into the oral cavity.

either pulmonary or oral. The mice in these groups appeared healthy throughout the study and no deaths occurred. Their fur was groomed and skin showed good turgor upon scruffing of the neck region during oral dosing, indicating adequate hydration. No diarrhea was noted in either of the SFL dosed groups.

3.3. Steady state trough serum levels

The average trough serum levels on days 3, 8, and 12 for each of the groups studied are shown in Fig. 4. From this figure, the average serum trough concentrations (μg/mL) for ITZ-pulmonary, Sporanox oral solution, and ITZ-oral on day 3 were 0.12, 0.31, and 0.16; on day 8 were 0.11, 0.37, and 0.26; and on day 12 were 0.11, 0.39, and 0.29, respectively. The Sporanox oral solution serum concentrations were significantly greater than the other groups on all days ($p < 0.05$). On day 3, the serum levels achieved by ITZ-oral and ITZ-pulmonary were not statistically different; however, on days 8 and 12, the ITZ-oral serum concentrations were significantly greater than ITZ-pulmonary ($p < 0.05$).

3.4. Steady state trough lung tissue concentrations

The average trough lung tissue concentrations on days 3, 8, and 12 for each of the groups studied are shown in Fig. 5. From Fig. 5, the average lung tissue concentrations (μg/g lung tissue) for ITZ-pulmonary, Sporanox oral solution, and ITZ-oral on day 3 were 2.16, 0.19, and 0.16; on day 8 were 2.22, 0.15, and 0.23; and on day 12 were 2.52, 0.18, and 0.15, respectively. ITZ-pulmonary achieved significantly greater (>10-fold) lung tissue concentrations compared to the Sporanox oral solution and ITZ-oral. There were no statistical differences between the two oral formulations which were dosed.

3.5. Lung-to-serum ratios

The ratio of lung-to-serum concentrations achieved through oral and pulmonary dosing was calculated from

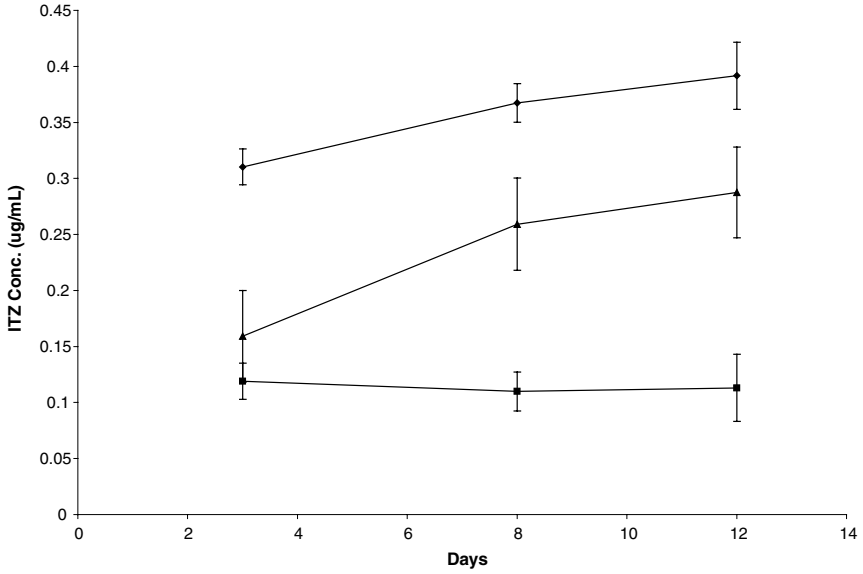

Fig. 4. Average serum concentrations for mice dosed with ITZ-oral (▲), Sporanox® oral solution (♦) or ITZ-pulmonary (■).

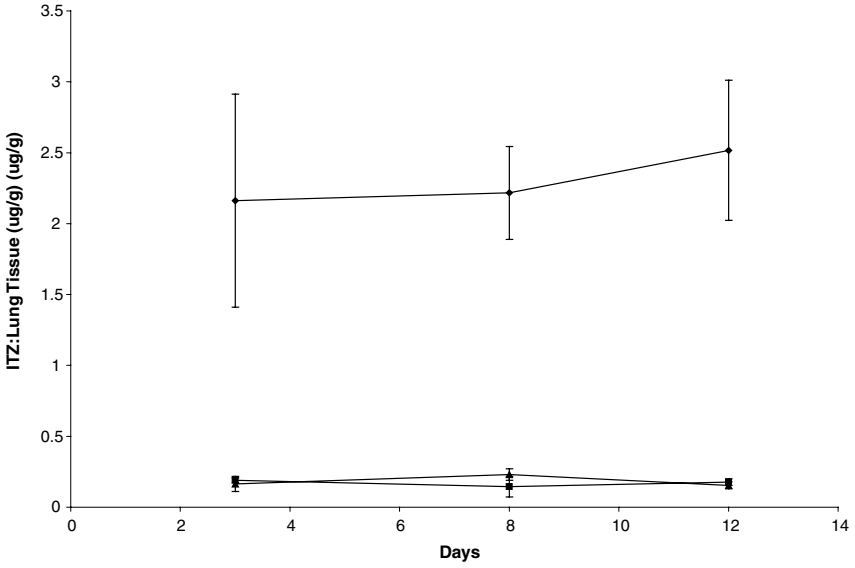

Fig. 5. Average lung tissue ITZ concentrations in mice dosed with ITZ-oral (▲) Sporanox® oral solution (■) or ITZ-pulmonary (♦).

Table 3
Average lung and serum trough levels in mice dosed with ITZ-oral, ITZ-pulmonary, and the Sporanox® oral solution which were used to calculate the lung:serum ratios for each group and time point

	Average lung concentrations (μg/g)			Average serum concentrations (μg/mL)			Lung:serum ratio			Reference lung:serum ratios	
Day:	3	8	12	3	8	12	3	8	12	Mice[a] [16]	Human[b] [17]
Amorphous ITZ: pulmonary	2.16	2.2	2.52	0.12	0.11	0.11	18.15	20.18	22.27		
Amorphous ITZ: oral	0.16	0.23	0.15	0.16	0.26	0.29	1.03	0.89	0.54		
Sporanox® oral liquid	0.19	0.15	0.18	0.31	0.37	0.39	0.61	0.40	0.45	0.28	3.7–12.2

Lung:serum ratios from other published studies are included in the table for comparison.
[a] Mice were dosed with 100 mg/kg Sporanox® oral solution once daily for 3 days. Lung and serum levels were measured 6 h after the last dose.
[b] Human subjects were dosed with 200 mg of Sporanox® oral solution or capsules twice daily until death ensued at which time the lung and serum ITZ was measured.

the measured values and is shown in Table 3. From the table, the lung:serum ratios for ITZ-pulmonary, Sporanox oral solution, and ITZ-oral on day 3 were 18.15, 0.61, and 1.03; on day 8 were 20.18, 0.40, and 0.89; on day 12 were 22.27, 0.45, and 0.54, respectively. Pulmonary dosed amorphous nanoparticles of ITZ (ITZ-pulmonary) achieved significantly greater lung levels per unit serum concentration compared to the orally dosed ITZ compositions. The data suggest that in a murine model, the partitioning of ITZ is greater in the circulatory system than in the lung tissue following oral absorption, except for day 3 of ITZ-oral. However, significant amounts of ITZ are retained in the lung tissue, compared to the circulatory compartment for mice which were dosed via inhalation.

4. Discussion

The present studies indicate that pulmonary dosing of ITZ is an effective mode of delivery for antifungal therapy and is able to achieve significant and sustained levels in the lung tissue. It was found that the concentrations which must be delivered via the pulmonary route are significantly lower than what is required orally, to achieve therapeutic serum and lung levels in vivo. Absolute bioavailability of the oral capsule is 55% in the fed state and 40% lower in the non-fed state [12]. The bioavailability is further reduced (40% of absolute bioavailability) when co-administered with a histamine-2-receptor (H_2)-antagonist. Also, patients with certain disorders (e.g., human immunodeficiency virus, HIV), which causes the stomach to have low gastric acidity (hypochlorhydria), showed high variability of absorption, due to poor dissolution of the drug. Co-administration of an acidic soda beverage is recommended in patients who use the capsule formulation of itraconazole [24]. The bioavailability of Sporanox oral solution, taken in a fasting condition, is approximately 60% higher than that of the capsules taken with a meal. However, hypochlorhydria also decreases oral absorption of the solution [12]. Dosing directly to the lung tissue eliminates many of the problems (pH dependence) with variability in absorp-

tion due to variability in gastric conditions, while achieving relatively high (>MLC) serum concentrations and extensive pulmonary concentrations, which remain above the MLC over a 24 h period.

In this study, the mice were exposed to 30 mg/kg through inhalation and dosed orally with 30 mg/kg by gavage. Although the Sporanox oral solution and ITZ-oral displayed significantly higher serum levels than ITZ-pulmonary, the pulmonary group sustained serum levels above 100 ng/mL at the trough. This is well above the suggested MLC determined by Allendoerfer et al. (70 ng/mL) [16]. The ultimate goal is enabling the ability to achieve a lower C_{max} (reduced toxicity) with extended time above the MLC for the organism. Furthermore, ITZ-pulmonary achieved nearly 10 times the lung tissue concentrations of the orally dosed ITZ at the trough.

Dosing of the Sporanox oral solution elicited several symptoms associated with toxicity. It is well documented that toxicity due to oral administration of cyclodextrins is high and mainly due to significant gastrointestinal side effects, such as diarrhea [25]. This significantly limits the dose which can be administered for the treatment of invasive fungal infections. These side effects were noted in the group dosed with the Sporanox oral solution. Dehydration, secondary to diarrhea, was noted in all of the animals in that group. Second, there were two deaths associated with toxicity, probably due to lethal diarrhea. Decreased grooming of the Sporanox oral solution group was also noted. This evidence of generally poor health observed among this group can be attributed to the dosing of the commercial cyclodextrin containing formulation of ITZ. It has been reported in human studies that diarrhea is associated with administration of Sporanox oral solution [26]. The degree and frequency of diarrhea was dose dependent and due to osmotic effects caused by the cyclodextrin component of the formulation. Local delivery of ITZ to the lung tissue should minimize systemic side effects while maintaining serum concentrations above the minimum lethal concentration (MLC) at the site of infection and eliminate the need for formulation with cyclodextrin.

Serum and lung tissue trough levels following multiple dosing of ITZ have never been reported in a murine model. Allendoerfer et al. [16] evaluated median steady state concentrations of ITZ in a murine model. Twelve hours following once daily dosing for 3 days of 100 mg/kg Sporanox oral solution, they measured serum levels as high as 9 μg/mL via a bioassay and a lung concentration of 2.5 μg ITZ/g of wet lung tissue. The ratio of lung:serum concentrations for the Allendoerfer et al. study was much lower than what was found in the present study for orally dosed Sporanox oral solution on day 3 (0.28 compared to 0.61), although the lung concentrations were significantly higher than what was required for effective treatment, based on an MLC of 70 ng/mL. Furthermore, the previous study found that lung tissue levels as high as 2.5 μg/mL were not nearly sufficient to prevent death in mice infected with *A. fumigatus*.

Studies in our laboratory have found that administration of ITZ-pulmonary significantly enhanced survival of mice which were infected with *A. fumigatus* [18]. Similar to Allendoerfer et al. [16], the current study found that Sporanox oral solution did not significantly enhance the survival of the mice in this infection model. Interestingly, the lung tissue concentrations achieved by Allendoerfer et al. through dosing of 100 mg/kg are similar to what was achieved in the current study through pulmonary dosing of only 30 mg/kg, although survival was significantly enhanced in the case of pulmonary administration [18]. It is evident that, although similar lung tissue concentrations can be achieved by both oral and pulmonary dosing, significantly higher doses of ITZ are required by oral administration and survival is greater when dosed via inhalation. This would be due to better distribution into the airway and interstitial spaces of the lung tissue as well as distribution to areas which may have low blood circulation because of fungal infiltration. It is also possible that toxicity due to dosing of 100 mg/kg Sporanox oral solution could have impacted the survival rates achieved by Allendoerfer et al. Toxicity due to diarrhea and dehydration was noted in the current study with only 60 mg/kg given daily in two divided doses.

Infection models using mice are cost effective and repeatable for the study of *Aspergillus* infections and their treatments. However, the distribution of ITZ may be vastly different between human and murine subjects. A study comparing lung and serum ITZ concentrations in patients which died as a result of aspergillosis was conducted by Coronel et al. [17]. Their study found that human patients display a higher lung:serum ratio for orally dosed ITZ, than was found in the current study. The pharmacokinetic mechanisms for distribution from serum into lung tissue are theorized to be different in mice than in humans, as is evident by a lung:serum ratio of less than one in the orally dosed mice compared to lung:serum ratios of greater than 3 in the human subjects. Coronel et al. suggested that, although the lung levels were high in these patients, their prognosis for survival did not improve. As a result, the patients died due to respiratory failure or dissemination of the *Aspergillus* from the lungs to other organs. Also, ITZ concentrations were not measured in the infected areas of the lung tissue, which could be lower than other regions of the lung. This proves advantageous for dosing of pulmonary ITZ to human subjects. Our study has found that high lung tissue concentrations were achieved by nebulization, which, based on Coronel et al., would remain high in the lung tissue, due to higher partitioning compared to the mouse model.

The lung:serum ratios achieved through pulmonary dosing are a significant advantage in treating or preventing *Aspergillus* infections in patients at risk. The portal of entry for *Aspergillus* is primarily through the pulmonary route. Achieving rapid and sustained levels locally, while minimizing systemic side effects (lower serum C_{max}), is an optimum criterion for prophylaxis. Based on the murine model,

significant ITZ levels are achieved after only 3 days of twice daily dosing of ITZ via inhalation.

5. Conclusion

Pulmonary dosing of ITZ compositions is an effective method for delivery of antifungal therapy for the treatment and prophylaxis of invasive fungal infections. High and sustained lung tissue concentrations were achieved via inhalation of an amorphous nanoparticulate ITZ-pulmonary composition while maintaining serum levels which are above the MLC of *A. fumigatus*. The lung:serum ratio was significantly greater in pulmonary dosed ITZ and could prove to enhance treatment while reducing side effects, by decreasing the systemic C_{max} and maintaining serum levels above the MLC. By enhancing the local delivery and reducing the potential for side effects of ITZ delivery, this delivery method can greatly improve morbidity and mortality for patients who are at risk of or infected with life-threatening fungal infections.

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