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# Itraconazole IV nanosuspension enhances efficacy through altered pharmacokinetics in the rat

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**Abstract**

The goal of this research was to evaluate an intravenous itraconazole nanosuspension dosage form, relative to a solution formulation, in the rat. Itraconazole was formulated as a nanosuspension by a tandem process of microcrystallization followed by homogenization. Acute toxicity, pharmacokinetics, and distribution were studied in the rat, and compared with a solution formulation of itraconazole. Efficacy was studied in an immunocompromised rat model, challenged with a lethal dose of either itraconazole-sensitive or itraconazole-resistant *C. albicans*. Itraconazole nanosuspension was tolerated at significantly higher doses compared with a solution formulation. Pharmacokinetics of the nanosuspension were altered relative to the solution formulation. $C_{max}$ was reduced and $t_{1/2}$ was much prolonged. This occurred due to distribution of the nanosuspension to organs of the monocyte phagocytic system (MPS), followed by sustained release from this IV depot. The higher dosing of the drug, enabled in the case of the nanosuspension, led to higher kidney drug levels and reduced colony counts. Survival was also shown to be superior relative to the solution formulation. Thus, formulation of itraconazole as a nanosuspension enhances efficacy of this antifungal agent relative to a solution formulation, because of altered pharmacokinetics, leading to increased tolerability, permitting higher dosing and resultant tissue drug levels.


*Keywords:* Nanosuspension; Itraconazole; Antifungal; Candidiasis; Macrophage

## 1. Introduction

Opportunistic fungal infections have increased very significantly over the last 25 years, in both incidence and severity with associated mortality. This has occurred as patients' immunological systems have become increasingly compromised, in response to disease states such as AIDS, cancer, and diabetes, as well as the use of more aggressive medical and surgical strategies, including life-support systems, broad-spectrum antibiotics, high dose cytotoxic chemotherapy and organ transplantation (Pfaller and Wenzel, 2003). To meet this challenge, an armamentarium of anti-fungal agents has been developed, all of which have significant shortcomings (Kontoyiannis, 2001). Many of the drug related issues are exacerbated because fungal disease is usually not diagnosed per se but is, more often, suspected. This places more emphasis on the safety profile of drugs, especially in cases where drugs are used empirically or prophylactically, rather than for treatment of confirmed infection (Hughes et al., 1997). Thus, while amphotericin is effective for many fungal strains, its adverse event profile especially to kidneys reduces its potential use. By contrast, fluconazole has emerged as a relatively safe anti-fungal drug, albeit with a much narrower spectrum of activity. As a result, its use has increased significantly, accompanied, however, by the appearance of fluconazole-resistant candidal strains (Revankar et al., 1996; Wingard et al., 1992).

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This paper seeks to reconcile the need for less toxicity and yet wider spectrum of activity, with a drug delivery technology applied to itraconazole, a member of the triazole class. As precedent, successful application of liposome delivery technology to amphotericin B had resulted in a much safer agent, although renal toxicity is still an issue (Walsh et al., 2004). Itraconazole commends itself because of its long history of use and hence relative safety particularly for the kidneys, its broad spectrum of activity for candidiasis, and applicability to aspergillosis (Boogaerts et al., 2001). While the commercially available SPORANOX® (itraconazole) Injection I.V. does have a label warning (Ortho Biotech, 2001) against its use for renally impaired patients, this arises from the cyclodextrin-solubilizing agent, rather than from the drug itself. Hence, removal of the cyclodextrin agent and utilization of another technology for this water insoluble compound is required.

Itraconazole was selected also because of its well-defined quantitative correlation between its in vitro MIC activity and clinical performance in the treatment of candidiasis. For susceptible organisms (MIC < 0.125 μg/ml), 89% of patients are cured clinically provided that itraconazole plasma concentration is greater than 0.5 μg/ml. Under these circumstances, for resistant organisms (MIC > 1.0 μg/ml), only 65% are cured. The intermediate MIC range of 0.25–0.5 has been proposed as a category defined as "susceptible dependent upon dose", S-DD (Rex et al., 1997). This suggests that the ability to dose more drug safely, potentially raising relevant body levels above the MIC, may move more organisms from the resistant category to the S-DD category.

To address the issues of insolubility as well as the need to dose higher amounts safely, nanosuspension technology was utilized. This involves a tandem process of microcrystallization followed by homogenization to produce submicron sized, surfactant-coated, drug crystals, small enough to be injected intravenously (Rabinow, 2004). Because of the slow dissolution kinetics in blood, the particles distribute to the organs of the monocyte phagocytic system, from which molecular drug is released over a prolonged timeframe. The altered pharmacokinetic profile affects the amount of drug that can be dosed safely.

## 2. Materials and methods

### 2.1. Preparation of itraconazole nanosuspensions

The procedure, utilizing NANOEDGE Technology, has been described previously (Kipp et al., 2003). Briefly, within a sterilized 9-glove isolator, a solution of itraconazole (Wyckoff) and Poloxamer 188 (BASF) in *N*-methyl-2-pyrrolidone (ISP/Pharmasolve) was added to an aqueous solution of sodium deoxycholate (Sigma–Aldrich) and glycerin, to induce rapid crystallization. This suspension was then homogenized, solvent was removed by successive centrifugation cycles, and the suspension was rehomogenized, and filled into vials at a concentration of 10 mg/ml itraconazole. Mean particle size for multiple 12 liter batches, and $d_{99}$ (particle size below which 99% of the volume of the suspension lies) were measured with an Horiba LA920 laser light scattering analyzer. Zeta potential ($\zeta$) was determined with a ZetaPALS (Brookhaven Instruments, electrode AQ471), with a sample suspension matrix identical to that of the medium of the nanosuspension.

### 2.2. Acute dose toxicity study

Studies were conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) with the approval of the institution's Animal Care and Use Committee (ACUC). Seven to eight weeks old male Sprague–Dawley rats (HSD: Harlan Sprague Dawley®SD™) were studied with 3–10 animals per dosing group. Rats were individually housed in suspended stainless steel cages, and provided rodent feed and water ad libitum. SPORANOX® Injection (at 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, and 80 mg/kg) and itraconazole nanosuspension (at 20 mg/kg, 30 mg/kg, 80 mg/kg, 160 mg/kg, and 320 mg/kg) were administered IV via a caudal tail vein at approximately 1 ml/min. Immediately after infusion, catheters were flushed with approximately 0.2 ml of saline. Control rats were given physiological saline. Animals were observed for any adverse clinical signs immediately and approximately 1 h following infusion and daily thereafter for the duration of the study. Individual body weights were determined prior to infusion and on days 2, 4, and 7. On study day 7, surviving animals were anesthetized with isoflurane then euthanized by exsanguination. A gross necropsy was performed for all surviving rats, including examination of the external body surface, all orifices, thoracic cavity and contents, cranial cavity and contents, and abdominal cavity and contents.

### 2.3. Transmission electron microscopy (TEM)

A rat received a single IV dose of 320 mg/kg itraconazole nanosuspension, and was necropsied 7 days later. Selected spleens were analyzed by light microscopy and some fixed in formalin followed by 2% glutaraldehyde in PBS. It was then osmicated in 2% osmium tetroxide followed by dehydration through a graded ethanol series through 100% ethanol into acetone followed by embedment in Spurr's resin. Thin sections were cut on a Reichert Jung Ultracut E ultramicrotome and stained with 5% aqueous uranyl acetate and Sato's lead (Sato, 1968). Thin sections were imaged to film at an accelerating voltage of 60 kV on a JEOL 1200 EX TEM.

### 2.4. Pharmacokinetics

Pharmacokinetics was studied in 8-week-old male Sprague–Dawley rats, three per dose group per time point. Each rat was bled at up to three time intervals. SPORANOX® Injection at 5 mg/kg and 20 mg/kg and 1% itraconazole nanosuspension at 20 mg/kg, 40 mg/kg, and 80 mg/kg were administered IV via a catheter in a caudal tail vein at a rate of approximately 1 ml/min. Immediately after infusion and before removal from the vein, the catheter was flushed with 0.2 ml of 0.9% sodium chloride injection USP. Following the single dose

administration, peripheral blood was collected at different time points. At the time of collection, each rat was anesthetized with isoflurane, to effect, and blood was collected from the orbital plexus into Vacutainer brand blood collection tubes containing 0.048 ml of 7.5% EDTA (Becton Dickinson, Franklin Lakes, NJ). Samples were drawn 2 min post-dose, and after 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 96 h, 144 h, and 192 h. The tubes were placed on ice until centrifuged (15 min at 2100 × *g*, 4 °C) to collect plasma, which was transferred to two 0.5 ml Eppendorf microcentrifuge tubes and stored frozen at −70 °C. Following analysis, described below, pharmacokinetic (PK) parameters for itraconazole (ITC) and hydroxy-itraconazole (ITC-OH) were derived from mean plasma concentration–time curves using noncompartmental methods with WinNonlin® Professional Version 3.1 (Pharsight Corp., Mountain View, CA). The various PK parameters that were analyzed included maximum peak concentration of the drug in plasma ($C_{max}$), the time to reach maximum concentration ($t_{max}$), half life ($t_{1/2}$), area under the curve (AUC), clearance (CL), volume of distribution at steady state ($V_{ss}$) and mean residence time (MRT). Secondary peak concentration ($C_{2\,max}$), and its time of attainment ($t_{2\,max}$), were noted from the raw data for the nanosuspension.

### 2.5. Analysis of itraconazole and hydroxy-itraconazole in plasma samples

Blood was collected in tubes containing EDTA, centrifuged 15 min at 2100 × *g* at 4 °C and the plasma was removed and stored at −70 °C until tested. Stability of the analytes under these conditions was verified. A 200 μl aliquot of rat plasma was pipetted into a 1.5 ml polypropylene centrifuge tube to which an internal standard of dialkylated itraconazole (DSM Pharma Chemicals, South Haven, MI Inc.) had been added and the solvent evaporated. Three hundred microlitres of acetonitrile was added to the plasma and the tubes were thoroughly mixed on a vortex mixer. The tubes were centrifuged for 5 min, and the supernatant was analyzed by RP-HPLC. Itraconazole standards (DSM Pharma Chemicals Inc., South Haven, MI) were prepared in plasma and treated identically to the samples. The assays were carried out using an Agilent 1100 Quaternary Pump, Autosampler, Column Compartment and Multi-Wavelength Detector, with an Alltech Altima C18 column, 250 mm × 4.6 mm, 5 μm column. A mobile phase of 36% 5 mM sodium phosphate (pH 6.7), 58% acetonitrile and 6% methanol was run isocratically at 37 °C at a flow rate of 1.1 ml/min. Analytes were detected by UV at 263 nm, with a bandwidth of 10 nm and fluorescence with 263 nm excitation and 380 nm emission. An injection volume of 100 μl was used.

### 2.6. Inoculum preparation

A Saubroud dextrose agar (SDA) plate was streaked with *C. albicans* ATCC Number 90029 ($MIC_{80}$ = 0.03 μg/ml for itraconazole), and placed in a 32 °C incubator for about 40 h. On the day of dosing, a yeast suspension was prepared by picking colonies with size ≤1 mm diameter and suspending them in sterile saline. The number of yeast cells in the suspension was counted using a hemacytometer and microscope. The yeast concentration in the inoculum was adjusted with additional saline. A plate count using a spread plate method was performed to determine the actual colony count. Serially, 10-fold dilutions of the inoculum in saline were prepared, and then SDA plates were inoculated with 0.2 ml/plate of the selected dilutions in duplicate. The fluid in each plate was spread with a sterile spreader. The plates were incubated at 32 °C and the colonies were scored 2–3 days later.

### 2.7. Anti-fungal Efficacy Study A: susceptible strain 90029

Male Sprague–Dawley rats, aged 8 weeks, were from a colony certified to be free of evidence of known pathogens, and were quarantined 3 days prior to the study. They were housed three per cage in sterile shoebox cages with filter top lids, and were provided autoclaved rodent feed and sterile drinking water ad libitum except during dosing. For those rats that were immuno-suppressed, 20 mg/kg subcutaneous injection of prednisolone sodium succinate (Solu-Delta Cortef, Pharmacia Upjohn) was divided into two doses at least 4 h apart on the day before and the day of inoculation. In the nanosuspension treated animals a single IV inoculation of 1 ml of $2.5 \times 10^6$ cfu *C. albicans*/ml saline was administered via caudal tail vein. The SPORANOX® treated and no-treatment groups were inoculated with 1 ml of either $9.5 \times 10^6$ (non-immunosuppressed animals) or $3 \times 10^6$ cfu (immunosuppressed animals) *C. albicans*/ml saline. Immediately after injection and before removal from the vein, the catheter was flushed with 0.2 ml of 0.9% sodium chloride for injection, USP.

There were six rats in each nanosuspension, SPORANOX®, and no-treatment control group. Beginning 4 h after inoculation, each itraconazole nanosuspension treated animal received an IV injection once every other day for 10 days (i.e., days 1, 3, 5, 7, and 9) with doses of 20 mg/kg, 40 mg/kg, or 80 mg/kg. The test article was administered IV via a caudal tail vein at approximately 1 ml/min. Rats in the SPORANOX® groups were treated daily at 5 mg/kg or 20 mg/kg for the first 2 days; however, due to toxicity seen in the 20 mg/kg group, these rats were dosed at 10 mg/kg for the remaining 8 days. Immediately after infusion, the catheters of all animals was flushed with 0.2 ml of 0.9% sodium chloride for injection, USP. Body weights were taken periodically over the course of the study. Surviving rats were terminated 11 days after inoculation and the kidneys were collected, weighed and cultured for determination of *C. albicans* colony counts or analyzed for itraconazole and hydroxy-itraconazole concentration. Kidneys were collected from untreated control rats when a moribund condition was observed, including when an animal had a 20% body weight loss compared to the body weight at inoculation.

### 2.8. Anti-fungal Efficacy Study B: susceptible strain 90029

Study B was identical to Study A above with the following exceptions: the identical inoculum level ($3.2 \times 10^6$) was used for all animals; the effect of treatment delay of 4 h or 24 h following

inoculation was studied; and kidney tissues were homogenized using a Polytron PT3000 homogenizer versus manual pressing using a steel mesh for Study A (see below).

### 2.9. Anti-fungal Efficacy Study C: resistant strain 201974

Rats were immunosuppressed with 20 mg/kg subcutaneous injection of prednisolone sodium succinate (Solu-Delta Cortef, Pharmacia Upjohn) divided into two doses at least 4 h apart on the day before, the day of, and the day following inoculation. Thereafter, immunosuppressive doses were administered once daily. Rats were inoculated with $5.0 \times 10^6$ cfu/ml of *C. albicans*, ATCC Number 201794, Lot 934031($MIC_{80} = 16$ μg/ml for itraconazole), prepared as above, once IV. Beginning 24 h after inoculation, rats were IV treated once daily for 10 days with 10 mg/kg SPORANOX® or once every other day for 10 days with nanosuspension itraconazole 20 mg/kg, 40 mg/kg, or 80 mg/kg. Body weight was determined daily. Rats were terminated after 10 days of exposure, and determinations were made for fungal count level as described above for Study B, as well as for mortality.

### 2.10. Kidney samples

Using a spatula, the kidney halves designated for drug assay were minced in glass scintillation vials. Four milliliter of extraction solution (acetonitrile, 0.1% phosphoric acid and internal standard) was added and the kidneys homogenized using an UltraTurrax T8 Homogenizer. The contents of the vials were vortexed, centrifuged and the supernatant analyzed using the same procedure as used for the plasma samples.

The remaining half of each kidney was processed by pressing them against a sterile steel mesh in a petri dish containing a fixed amount of media or saline (Study A) or utilizing a Polytron homogenizer (Study B). The kidney suspension was serially 10-fold diluted and then selected dilutions were inoculated (0.2 ml/plate) onto SDA plates in duplicate. The fluid in each plate was spread with a sterile spreader. The plates were incubated at 32 °C and the colonies were scored 2–3 days later. The amount of yeast in each rat kidney sample was calculated as CFU/g. To probe the in situ fungistatic behavior of the formulation, no attempt was made to remove the drug from the kidney prior to homogenization to eliminate carryover effect.

### 2.11. Tissue distribution and mass balance

[$^3$H]-Itraconazole at a specific activity of 142 μCi/mg was synthesized and prepared as a nanosuspension at a concentration of 7.4 mg/ml. Seven weeks old Sprague–Dawley rats were dosed IV via tail vein over 2 min at a dose of 10 mg/kg. Tissues, urine, and feces were subsequently collected at time points ranging from 1 h to 504 h for radioactive determination of tissue distribution and mass balance. Specifically, three rats per time point were sacrificed via exsanguination (cardiac puncture) under halothane anesthesia at 1 h, 3 h, 5 h, 8 h, 24 h, 48 h, 96 h, 168 h, 336 h, and 504 h post-dose. At each time point, the tissue samples of 26 different organs were collected from three animals. From animals in a second group, urine was collected in plastic containers surrounded by dry ice at 0–8 h and 8–24 h post-dose, and at 24-h intervals through 504 h post-dose. Feces were also collected in plastic containers surrounded by dry ice at 24-h intervals through 504 h post-dose.

## 3. Results

### 3.1. Physical characterization

The zeta potential of the itraconazole nanosuspension was determined to be $-30.55 \pm 2.90$ mV. For four 12 liter batches, mean particle size and standard deviation (S.D.) measured at 5 °C over 24 months was $0.581 \pm 0.018$ μm. Over this same interval the $d_{99}$ and S.D. was $2.234 \pm 0.094$ μm. At 25 °C over 6 months, mean particle size and S.D. were $0.620 \pm 0.040$ μm and $d_{99}$ and S.D. was $2.310 \pm 0.291$ μm. Also stable were pH (7.5) and osmolality (258 mosmol/l).

### 3.2. Acute toxicity

All animals treated with 40 mg/kg and 80 mg/kg, and one animal of five treated with 30 mg/kg SPORANOX®, died within a few minutes of IV dosing or were euthanized because they were moribund (Table 1). Animals dosed with 30 mg/kg SPORANOX® showed ataxia, labored respiration and lethargy, immediately after treatment but these symptoms disappeared in surviving animals within an hour. Two of five animals in the 20 mg/kg SPORANOX® group showed slight lethargy and irregular breathing, but this disappeared by the following day. Some animals in groups that received 160 mg/kg and 320 mg/kg of nanosuspension showed signs of red ears and/or feet at 1 h after treatment. This disappeared by the following day. No group differences were apparent for body weight gain over the 7-day duration of the study for the 0 mg/kg, 5 mg/kg, and 10 mg/kg SPORANOX® groups and the 40 mg/kg and 80 mg/kg nanosuspension groups. However, there was a decreased body weight gain over the 7-day period for the 20 mg/kg and 30 mg/kg SPORANOX® groups and the 320 mg/kg nanosuspension group. The surviving SPORANOX® and 40 mg/kg, 80 mg/kg, and 160 mg/kg nanosuspension groups had no treatment-related gross necropsy findings. A finding of pale, enlarged spleen was noted in all 10 animals treated with a dose of 320 mg/kg nanosuspension. A microscopic evaluation revealed splenic cytoplasmic enlargement and vacuolation of macrophages. Crystalline material was clearly evident in the macrophages of a transmission electron micrographic section of the spleen of a rat that had received itraconazole nanosuspension (Fig. 1). For nanosuspensions, the no observed effect level, or NOEL, was 80 mg/kg. NOEL is defined as the highest dose that had no effect on any of the measured parameters in any of the studies.

### 3.3. Pharmacokinetics

As dose of SPORANOX® increased from 5 mg/kg to 20 mg/kg, $AUC_{(0-\infty)}$ values of itraconazole increased more than

Table 1
Clinical observations in acute toxicity study

| Treatment | Itraconazole dose (mg/kg) | Incidence of death/number of animals | Immediate observations (within 1 h of administration, day 0) | Delayed observations (days 1–7 post-injection) |
|---|---|---|---|---|
| Saline | 0 | 0/10 | None | None |
| Nanosuspension | 40 | 0/10 | None | None |
| Nanosuspension | 80 | 0/10 | None | None |
| Nanosuspension | 160 | 0/10 | None | 3 of 10 Red feet[a] |
| Nanosuspension | 320 | 0/10 | None | 5 of 10 Red feet[a] |
| SPORANOX® | 5 | 0/6 | None | None |
| SPORANOX® | 10 | 0/6 | None | None |
| SPORANOX® | 20 | 0/5 | Two of five slight lethargy and irregular breathing | None |
| SPORANOX® | 30 | 1/5 | Five of five lethargy, irregular breathing, and ataxia | None |
| SPORANOX® | 80 | 3/3 | Three of three irregular respiration, two of three gasping, one of three convulsions | None |

[a] Occurred in the second set of five animals and may have been inadvertently overlooked in the first set of five animals.

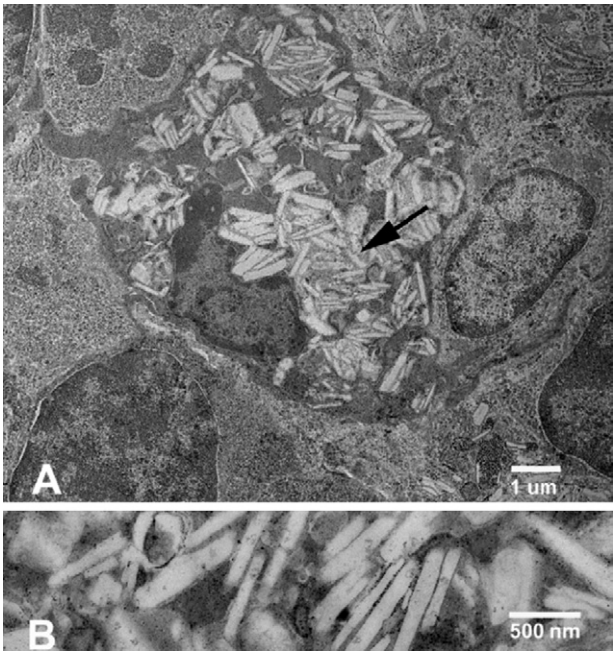


Fig. 1. TEM of spleen of rat, dosed IV with itraconazole nanosuspension, showing crystalline material in macrophages.

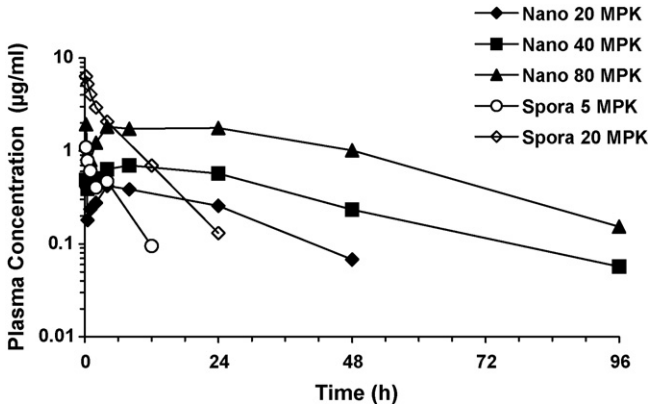


Fig. 2. Plasma itraconazole (ITC) concentration time profiles following injection of itraconzole nanosuspension and SPORANOX®.

dose proportionately, while plasma clearance, CL, correspondingly decreased substantially (Table 2, Fig. 2). The apparent half-life of itraconazole for the terminal phase at the 20 mg/kg dose was greater than the half-life at the 5 mg/kg dose level. For the hydroxy-itraconazole metabolite, on the other hand, $AUC_{(0-\infty)}$ increased nearly proportionally with SPORANOX® dose, while the half-life remained nearly the same (Table 2).

As observed with SPORANOX® solution, the dose dependent pharmacokinetics of itraconazole in the nanosuspension

Table 2
Pharmacokinetic parameters for itraconazole (ITC) and hydroxy-itraconazole (ITC-OH) in rats after a single IV administration of SPORANOX® (Spor) or itraconazole nanosuspension (Nano)

| Formulation/dose | Analyte | $t_{max}$ (h) | $C_{max}$ (μg/ml) | $t_{2\,max}$ (h) | $C_{2\,max}$ (μg/ml) | $AUC_{(0-\infty)}$ (μg h/ml) | $t_{1/2}$ (h) | CL (ml/h) | $V_{ss}$ (l/kg) | $MRT_{last}$ (h) |
|---|---|---|---|---|---|---|---|---|---|---|
| Spor5 | ITC | 0.03 | 2.18 | – | – | 3.94 | 2.84 | 317 | 4.94 | 2.58 |
| Spor20 | ITC | 0.03 | 11.92 | – | – | 29.6 | 5.05 | 169 | 3.88 | 4.65 |
| Nano20 | ITC | 0.03 | 20.3 | 4 | 0.420 | 16.5 | 15.6 | 303 | 30.8 | 13.4 |
| Nano40 | ITC | 0.03 | 57.6 | 8 | 0.698 | 41.9 | 17.2 | 239 | 37.7 | 24.4 |
| Nano80 | ITC | 0.03 | 218 | 4 | 1.82 | 140 | 20.8 | 143 | 26.0 | 25.8 |
| Spor5 | ITC-OH | 12 | 0.17 | – | – | 3.78 | 5.93 | – | – | 7.08 |
| Spor20 | ITC-OH | 4.0 | 0.78 | – | – | 14.2 | 5.68 | – | – | 11.9 |
| Nano20 | ITC-OH | 24 | 0.37 | 24 | 0.368 | 14.4 | 12.0 | – | – | 27.5 |
| Nano40 | ITC-OH | 24 | 0.61 | 24 | 0.61 | 30.2 | 22.3 | – | – | 37.8 |
| Nano80 | ITC-OH | 24 | 1.03 | 24 | 1.03 | 67.4 | 22.1 | – | – | 44.4 |

were also demonstrated over an IV dose range of 20–80 mg/kg (Table 2). The increase in $AUC_{(0-\infty)}$ values of itraconazole was more than dose proportional, comparing particularly the two highest doses. Half-lives, however, were similar for these two highest doses. Clearance decreased with dose. Overall, the pharmacokinetic parameters including $t_{1/2}$ and mean residence time, MRT, indicate that itraconazole concentrations in the nanosuspension were sustained much longer when compared with SPORANOX® solution. The apparent plasma half-life of itraconazole administered as nanosuspension was 15.6 h (S.D. 2.9) at the 20 mg/kg dose, whereas the half-life was 5.05 h (S.D. 0.53) when given as SPORANOX® solution at an equivalent dose. However, the plasma CL for itraconazole administered as nanosuspension was 303 ml/h (S.D. 23) compared with 169 ml/h (S.D. 30) for the solution. For the hydroxy-itraconazole metabolite, $AUC_{(0-\infty)}$ increased nearly proportionally with nanosuspension dose, while $t_{1/2}$ tracked closely with that of the parent compound (Table 2).

Peak plasma concentrations of itraconazole following IV administration of nanosuspension were seen at the earliest time-point of analysis. Following a decrease from this $C_{max}$, the concentration starts to increase after about 30 min (Fig. 2). A secondary peak, designated as $C_{2\,max}$, began to appear at time points designated as $t_{2\,max}$, 4–8 h after administration, and is tabulated in Table 2.

The dose level of 20 mg/kg was common to the SPORANOX® and nanosuspension groups. At this dose, the plasma concentration of SPORANOX® remained higher than 1 μg/ml during the first 8 h, whereas the nanosuspension remained below 0.5 μg/ml throughout the entire time course. Despite the longer half-life of the nanosuspension, its $AUC_{(0-\infty)}$ was about half that of SPORANOX®, 16.5 versus 29.6, at the common dose of 20 mg/kg at which both may be compared.

The ratio of $AUC_{(0-\infty)\ itraconazole}/AUC_{(0-\infty)\ hydroxy\text{-}itraconazole}$ approached 1 for the lowest doses of both SPORANOX® dose and nanosuspension. As dose increased for both formulations, the ratio exceeded one.

### 3.4. Tissue distribution and mass balance

By 504 h (21 days) following administration, mean mass balance of radioactivity was 88.2%. Tissue concentrations of radiolabeled itraconazole (μg equiv./g) are plotted versus time post-dose for particular tissues in Fig. 3. The highest levels appear in the liver and spleen, where concentrations are highest initially and decline thereafter. Levels in the fat are lower initially, increase to a maximum at about 8 h, and then decline subsequently. Kidneys reflect a similar, though less pronounced, biphasic behavior. Plasma concentrations are somewhat higher than but comparable to blood.

### 3.5. Efficacy studies

All drug-treated rats in Efficacy Study A gained weight and survived over the course of the study (data not shown). The untreated rats consistently lost weight after inoculation and five of six rats died or became moribund and were terminated by day 7. One rat survived the inoculation and began gaining weight after day 7. The weight gain for 80 mg/kg nanosuspension rats was slightly less than for the 20 mg/kg and 40 mg/kg. No observable abnormalities were exhibited throughout the duration of the study for nanosuspension rats.

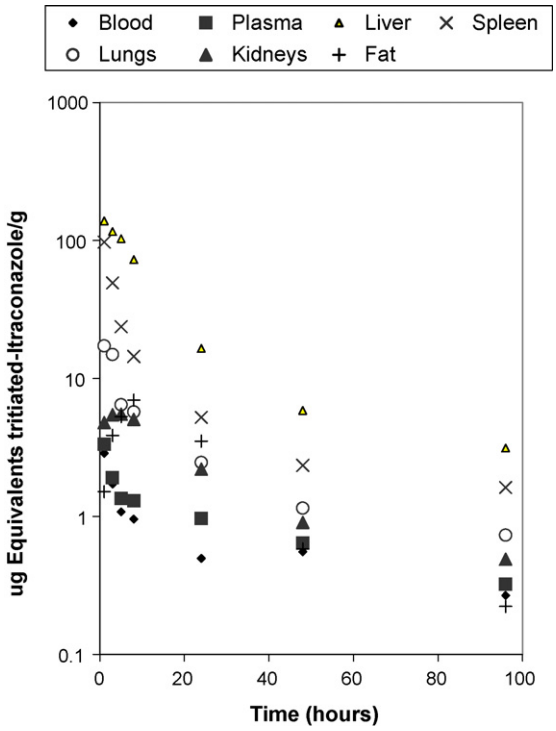


Fig. 3. Concentration of radioactivity in tissues post-dosing with IV itraconazole nanosuspension 10 mg/kg.

Treatment with SPORANOX® at both the low and high dose in Study A resulted in a 3–4 log reduction in kidney *C. albicans* titer compared to the no treatment groups (Table 3). However, there were no statistically significant differences among the four groups treated with SPORANOX® at dosages of 5 mg/kg or 10–20 mg/kg, regardless of whether immunosuppressant was used or not. In the immunosuppressed group, the incidence of infection was more consistent with 6/6 and 4/6 animals having similar *C. albicans* colony counts after low and high SPORANOX® treatment, respectively, compared to only 1/6 and 2/5 for the corresponding non-immuno-suppressed groups.

Treatment with nanosuspension in Study A resulted in protection of the animals from infection (zero recoverable counts in all animals) for the higher dosages of 40 mg/kg and 80 mg/kg. Attendant with this result were considerably higher drug levels in the kidney, 18.5 μg/g and 41.2 μg/g, for the higher doses of nanosuspension, than was the kidney drug level of 8.5 μg/g for SPORANOX administered at its highest doses studied. For the nanosuspension groups in Study A, kidney itraconazole levels increased in a dose dependent manner, 6.1 for 20 mg/kg, 18.5 at 40 mg/kg, and 41.2 at 80 mg/kg. Pairwise comparisions showed each dose was statistically different from the other ($p < 0.1$ for all comparisions, Table 3).

In Study B, a significant difference between colony counts for SPORANOX® and nanosuspension groups was apparent only

Table 3
Efficacy Study A: susceptible strain. Mean *C. albicans* kidney colony count and itraconazole (ITC) and hydroxy-itraconazole (ITC-OH) concentration

| Treatment | *C. albicans* titer | | Concentration in kidney | |
|---|---|---|---|---|
| | Mean count (cfu/g) | Incidence of non-zero recoverable counts | ITC (μg/g) | ITC-OH (μg/g) |
| Immunosuppressed (inoculum: $3 \times 10^6$ cfu/ml) | | | | |
| No treatment | $(7.4 \pm 2.8) \times 10^4$ | 6/6 | – | – |
| SPORANOX®, 5 mg/kg | $96.7 \pm 13.2$ | 6/6 | $1.15 \pm 0.08$ | |
| SPORANOX®, 10–20 mg/kg | $12.3 \pm 13.1$ | 4/6 | $8.5 \pm 2.3$ | |
| No suppression (inoculum: $9.5 \times 10^6$ cfu/ml) | | | | |
| No treatment | $1.5 \times 10^5$ | 6/6 | – | – |
| SPORANOX®, 5 mg/kg | 158.8 | 1/6 | $0.0 \pm 0.1$ | $0.2 \pm 0.1$ |
| SPORANOX®, 10–20 mg/kg | 86.1 | 2/5 | $0.4 \pm 0.3$ | $1.0 \pm 0.3$ |
| Immunosuppressed (inoculum: $2.5 \times 10^6$ cfu/ml) | | | | |
| No treatment | $(3.5 \pm 5.81) \times 10^5$ | 6/6 | – | – |
| Nanosuspension, 20 mg/kg | $5.0 \pm 5.1$ ($n = 6$ two $N$ values as 0) | 4/6 | $6.1 \pm 4.6$ | $5.7 \pm 3.7$ |
| Nanosuspension, 40 mg/kg | 0 | 0/6 | $18.5 \pm 4.0$ | $6.0 \pm 0.6$ |
| Nanosuspension, 80 mg/kg | 0 | 0/6 | $41.2 \pm 16.0$ | $6.2 \pm 1.0$ |

Table 4
Efficacy Study B: susceptible strain. Mean *C. albicans* kidney colony count and itraconazole (ITC) and hydroxy-itraconazole (ITC-OH) concentration. All immunosuppressed, inoculum: $3.2 \times 106$ cfu/ml

| Treatment | Treatment delay (h) | *C. albicans* titer | | Drug concentration in kidney | |
|---|---|---|---|---|---|
| | | Mean count (cfu/g) | Incidence of non-zero recoverable counts | ITC (μg/g) | ITC-OH (μg/g) |
| No treatment | | $2.7 \times 10^6$ | 6/6 | – | – |
| SPORANOX®, 10 mg/kg | 4 | 86.9 | 6/6 | $0.4 \pm 0.11$ | $0.9 \pm 0.15$ |
| SPORANOX®, 10 mg/kg | 24 | 380.3 | 6/6 | $0.5 \pm 0.18$ | $1.1 \pm 0.30$ |
| Nanosuspension, 40 mg/kg | 4 | 59.6 | 4/6 | $12.8 \pm 5.7$ | $2.9 \pm 0.4$ |
| Nanosuspension, 40 mg/kg | 24 | 33.6 | 6/6 | $13.1 \pm 7.0$ | $2.2 \pm 0.16$ |
| Nanosuspension, 80 mg/kg | 24 | 1.9 | 1/6 | $64.3 \pm 35.4$ | $2.8 \pm 0.51$ |

for the more severe group involving the 24 h treatment delay (Spor 10 mg/kg versus nano 40 mg/kg: $p < 0.001$; Spor 10 mg/kg versus nano 80 mg/kg: $p < 0.0001$) (Table 4). Kidney levels of itraconazole were consistent for the same dose of 10 mg/kg SPORANOX® comparing the 4 h and 24 h treatment delays, at 0.4 μg/g and 0.5 μg/g, respectively. Similarly kidney levels of itraconazole were consistent for the same dose of 40 mg/kg nanosuspension comparing the 4 h and 24 h treatment delays, at 12.8 μg/g and 13.1 μg/g, respectively. Tissue levels increased with nanosuspension dose.

Study C constitutes a very severe model, involving daily immunosuppression, and an itraconazole-resistant Candida strain, where the key test parameter is survival (Fig. 4). In contrast with the SPORANOX® animals in which all died during the study, at least half the animals survived if treated with nanosuspension. The combined groups of 20 mg/kg, 40 mg/kg, and 80 mg/kg nanosuspension formulation resulted in greater survival than did SPORANOX® IV ($p < 0.01$, Kaplan–Meier estimate using log-rank statistics calculated with SAS PROC Lifetest, v. 9.1).

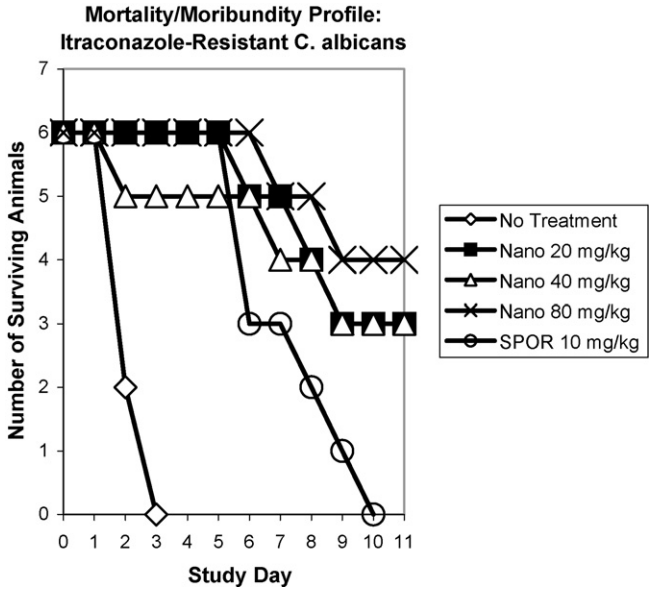


Fig. 4. Mortality/moribundity profile after 10 days dosing daily with SPORANOX® (SPOR) 10 mg/kg or every other day with itraconazole nanosuspension (Nano) at 20 mg/kg, 40 mg/kg, or 80 mg/kg in rats systemically infected with itraconazole-resistant *C. albicans*.

## 4. Discussion

Rats tolerate itraconazole nanosuspension significantly better than they do SPORANOX® cyclodextrin solution of itracona-

zole, as shown in Table 1. No mortality was seen at the highest dose of 320 mg/kg studied for the nanosuspension formulation, whereas SPORANOX® resulted in death of some of the animals at as low a dose as 30 mg/kg. This difference is attributable to altered pharmacokinetics, Fig. 2, resulting in a much reduced $C_{max}$ for the nanosuspension. In support of this hypothesis, a rapidly dissolving amorphous nanosuspension of itraconazole, with a high $C_{max}$ similar to that of SPORANOX®, caused lethality in half the study rats at 80 mg/kg and all the rats at 160 mg/kg (Baxter Healthcare Corp., unpublished data). In this case, having all of the drug systemically available simultaneously may exceed permissible levels of safety. These data imply that there may be a significant benefit to using a non-dissolving nanosuspension formulation of insoluble compounds, in the case where toxicity is mediated by $C_{max}$ levels. These findings are consistent with the similarly high doses of itraconazole that are tolerated orally ($LD_{50} > 320$ mg/kg) in the rat (Van Cauteren et al., 1987), presumably also because of prolonged low level absorption.

Alternatively, the greater tolerability of the nanosuspension may be related to lower toxicity of the excipients used in the nanosuspension relative to the excipients in SPORANOX®. However, this is not consistent with the known safety data of the key excipient, 2-hydroxypropyl-beta-cyclodextrin (400 mg/ml), in SPORANOX®. The lethal dose of this excipient was found to be greater than 5 g/kg when administered intravenously in the mouse (Sharma et al., 1995). Cynomologus monkeys were able to tolerate 10 g/kg acute exposure with no lethality (Brewster et al., 1990). These values are considerably in excess of the calculated 1.2 g/kg dose of 2-hydroxypropyl-beta-cyclodextrin associated with the administration of the lethal dose of 30 mg/kg SPORANOX® in rats. Hence the increased tolerability of the nanosuspension appears to be more consistent with modified pharmacokinetics rather than changed excipients.

After intravenous administration, slowly dissolving nanosuspensions distribute initially to organs of the monocyte phagocytic system (MPS), particularly the spleen and liver, Fig. 3, where they are histologically evident (Fig. 1). Subsequent dissolution provides a depot effect. The finding of initial sequestration by the MPS, followed by slow release is generally found for intravenously administered nanoparticulate dosage forms (Moghimi et al., 2001).

Alternative ways of sequestration of nanoparticles in the organs of the MPS, other than by phagocytosis, are certainly possible. However, histological analysis revealed that drug particle accumulation was apparent only in the macrophages, within the cytoplasm. Drug was not identified anywhere else. The resident macrophages not only contain the drug but increase in number with increasing dose to accommodate the particle burden, as shown by results of subchronic rat toxicity studies not reported in this paper.

Relatively high peak plasma levels are initially seen minutes after IV administration of nanosuspension, Fig. 2, but quickly decrease. This initial $C_{max}$ is mostly due to nanosuspension particles present in vivo at high levels at early timepoints inadequately removed from the plasma by centrifugation (data not presented). Sufficiently frequent sampling of plasma in the initial hours following administration is required to observe the secondary peak maxima, $C_{2\,max}$ at 4–8 h in Fig. 2. This maximum in the plasma level is believed to arise from a maximum in the release rate of drug from the organs of the MPS. It is consistent with the sharp decrease in the spleen concentration seen at this time (Fig. 3).

The more than dose proportional increases in itraconazole $AUC_{(0-\infty)}$ has been explained by transient saturation of the metabolic processes in the liver (Heykants et al., 1987; Heykants et al., 1989). A similar explanation can be made for the decreasing value of $AUC_{(0-\infty)\ hydroxy\text{-}itraconazole}/AUC_{(0-\infty)\ itraconazole}$ with dose for either SPORANOX® or nanosuspension.

The longer half-life for the nanosuspension permitted reduction of dosing frequency to once every 2 days in these studies. Because itraconazole was shown to be more tolerated in a nanosuspension than in a solution formulation, increased amounts could be administered in the efficacy studies, resulting in higher kidney levels (Tables 3 and 4). In Efficacy Study A, an itraconazole sensitive strain of *C. albicans*, with MIC = 0.03 μg/ml was used. While treatment with SPORANOX® decreased the counts considerably, treatment with nanosuspension resulted in even fewer recoverable fungal colonies (Table 3). This behavior correlated well with the kidney drug levels, which in turn increased with dose.

It should be noted that $C_{max}$ of SPORANOX® is significantly greater than that for treatment with nanosuspension (Fig. 2). Despite this, nanosuspension treatment was more effective in reducing colony counts. This is consistent with the finding that the pharmacological parameter that best correlates with efficacy for triazole antifungal agents for candidiasis is AUC/MIC, rather than $C_{max}$/MIC (Schafer-Korting et al., 1991; Schafer-Korting et al., 1995; Andes and van Ogtrop, 1999; Andes, 2003; Andes et al., 2003, 2004).

There is a difference in colony counts for the non-treated control, between the immunosuppressed and non-immunosuppressed groups in Efficacy Study A. This is indicative of the synergistic therapeutic interactions between antifungal agents and the host cellular immune system (Van't Wout et al., 1990; Brummer et al., 1991; Xhonneux et al., 1992; Garcha et al., 1995; Kullberg, 1997; Hara et al., 1998; Stevens, 1998).

To explore further the effect of drug level in an immunocompromised animal, anti-fungal effect was studied in a more severe model in Efficacy Study B. Although the same sensitive strain was used, a more onerous 24 h treatment delay was studied in addition to the same 4 h delay as used in Efficacy Study A. Additionally, the efficiency of extracting fungal colonies from kidney tissue was improved with the implementation of a homogenizer in place of the manual grinding. This led to increased fungal counts for the no-treatment control relative to that for the earlier study ($2.7 \times 10^6$ versus $3.5 \times 10^5$). Counts were increased for the 4 h delay SPORANOX® and nanosuspension groups as well. Nevertheless, in the more severe 24 h delay, the nanosuspension decreased colony counts significantly more than did SPORANOX® (33.6 versus 380.3; $p < 0.001$) (Table 4). This was associated with greater drug concentrations in the kidneys.

The severity of the animal model was increased still further in Efficacy Study C. This utilized, in addition to a 24 h delay

between inoculation and start of treatment, daily immunosuppression, and most importantly, an itraconazole-resistant strain with $MIC_{80} = 16$ μg/ml for itraconazole. This level of resistance far exceeds the recommended guideline of MIC < 0.125 μg/ml for reasonable expectation of successful treatment outcome with itraconazole. As a result the no-treatment group incurred an order of magnitude increase in the fungal count by the end of the study (data not shown). In view of these adverse factors, not surprisingly, all of the animals in the SPORANOX® group died by the end of the study (Fig. 4). However, the animals in the nanosuspension groups survived statistically longer ($p = 0.01$).

## 5. Conclusions

The safety, pharmacokinetics, tissue distribution, and efficacy of an intravenous itraconazole nanosuspension dosage form, made by a tandem microprecipitation/homogenization process, was characterized in the rat. In comparison with a solution formulation of this drug, the nanosuspension was determined to be more tolerable at higher doses in a rat model. This was attributed to MPS deposition, resulting in altered pharmacokinetics, involving lower $C_{max}$ and longer $t_{1/2}$. The increased safety permitted higher dosing in rat efficacy models. This led to higher drug levels in the target organs, leading to reduced colony counts, and improved survival as severity of the model was increased. Enhancement of efficacy for other indications, such as cancer, may be anticipated for MPS targeting nanosuspensions whose toxicity is mediated by $C_{max}$ but efficacy is determined by AUC and prolonged half-life.

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